Synthesis of Novel C-2 Substituted Pyrimidine Nucleoside Analogs

Martin Dunkel [1], P. Dan Cook and Oscar L. Acevedo*

Medicinal Chemistry Department,
ISIS Pharmaceuticals, Inc., 2292 Faraday Avenue,
Carlsbad, CA 92008

Received June 7, 1993

Dedicated to the memory of Dr. Roland K. Robins

A series of 2-(2-oxoalkylidene)-4(1*H*)-pyrimidinone nucleoside analogs were synthesized by the addition of the lithium enolates of methylketones to 2,5′- and 2,2′-anhydrouridines and to 2,5′-anhydrothymidines. Alternatively, 2-thiouridine was alkylated with bromomethyl ketones to yield 2-(2-oxoalkyl)thio-4(1*H*)-pyrimidinone ribofuranosides in good yields. These intermediates were subsequently transformed into the title compounds *via* an Eschenmoser sulfur extrusion reaction. The 2-(2-oxoalkylidene)-4-(1*H*)-pyrimidinone nucleoside analogs exhibit enol proton signals in their ^{1}H nmr spectra indicative of hydrogen bonding between N-3 and keto oxygen. These structures offer functional groups with potential for Watson-Crick hydrogen bonding.

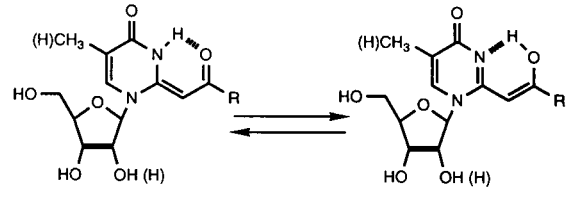
J. Heterocyclic Chem., **30**, 1421 (1993).

Introduction.

Recent history has witnessed a growing interest in the chemistry and biology of oligonucleotides which target cellular and viral mRNAs. These molecules, referred to as antisense oligonucleotides [2], are designed to inactivate their complements at the transcriptional or translational level *via* hybridization arrest or by the intervention of cellular RNase H enzymes. We envision an alternate approach employing reactive functionalities tethered to an antisense oligonucleotide and directed at specific centers on an RNA target. Specifically, targeting the N-3, C-1′ or 2′-OH of adenine would require positioning of these functionalities in the minor groove of an antisense DNA-mRNA heteroduplex. This requirement has prompted our synthesis of novel C-2 substituted pyrimidine nucleosides with the following general features: a) preservation of Watson-Crick hybridization characteristics b) stability during automated DNA synthesis and deprotection of oligomers and c) the conformational rigidity of a carbon tether to accurately position functionalities near their target.

Criteria a and b would not be met by the readily available 2-*O*-, -*S*- or *N*-alkylpyridimine nucleosides. For example, these heteroalkyl substituents would preclude the availability of an imino proton at N-3 that is necessary for hydrogen bonding. In addition, post-synthesis deprotection of DNA oligomers using concentrated ammonia would likely yield isocytidine and uridine from the displacement of the heteroalkyl substituents at C-2. Likewise, *N*-alkylated moieties at this position would fail criterion c due to the spatial instability inherent in the inversion of configuration of a secondary amine. A hypothetic 2-alkylpyrimidin-4-one nucleoside would be stable to nucleophilic displacement although it would also lack an N-3 proton. However, if the tether contained a keto group in a position β to the pyrimidine ring, enolization of this group would in principle provide the required proton, where this proton is bound by the N-3 of the pyrimidine and oxygen of the keto function (Figure 1). The specificity of these enolized

Figure 1



Structure of 2-(2-oxoalkylidene)pyrimidinone nucleoside analogs

pyrimidines for adenine is important, for it will determine the fidelity of our oligonucleotides to their RNA targets. Finally, though the 2-β-ketoalkylpyrimidin-4-one structures may satisfy all criteria listed above, they may share the acid lability of the glycosidic bond [3,4] of other pyrimidine nucleosides substituted with carbon at C-2. In order to address these and other questions, we have synthesized a variety of ribo-, arabino- and 2′-deoxyribofuranose pyrimidines substituted with carbon tethers at C-2. In this report, we describe the novel and facile synthesis of certain 2-(β-ketoalkyl)pyrimidin-4-one nucleoside analogs.

Results and Discussion.

Only a few reports describing the synthesis of 2-alkyl substituted pyrimidine nucleosides can be found in the literature. Rosenthal and Dodd [3] obtained 2-methylpyrimidin-4-one ribonucleosides from the reaction of 2,2′-anhydrouridines with 1,3-dithiane/*n*-butyllithium followed by desulfurization. In a related procedure, Kunieda and Witkop [4] reported that the treatment of a protected 2,5′-

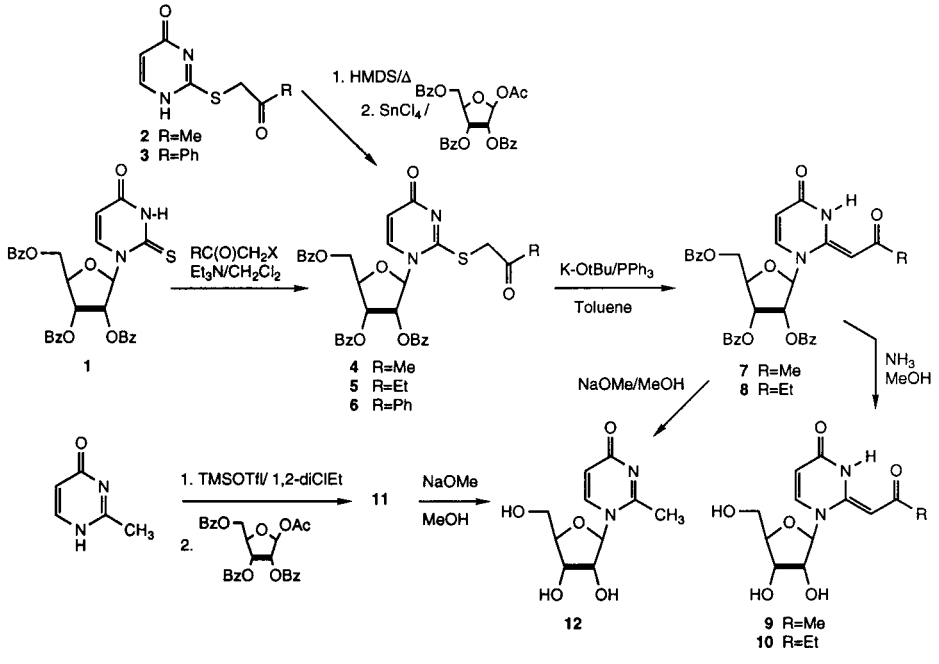
anhydrouridine with dimethylsulfoxonium methylide (DMSY) gave a 2-methylpyrimidin-4-one ribonucleoside after hydrogenolysis of the sulfoxonium group. Our attempts to synthesize a variety of 2-(β-ketoalkyl)pyrimidine nucleosides using DMSY chemistry centered around the possibility of acylating the intermediate sulfoxonium nucleosides with a variety of tethers [5]. Unfortunately, these acylations resulted in low yields and laborious purifications. Furthermore, reduction of the intermediate sulfoxonium group after acetylation invariably led to products of overreduction.

Vorbrüggen and Krolikiewicz [6] have reported that an Eschenmoser sulfur extrusion of 2-*S*-phenacylated-2-thiouridines yielded 2-phenacetylpyrimidin-4-one ribonucleosides. In this regard, we have alkylated 2-thiouridine (**1**) with 1-chloroacetone or 1-bromo-2-butanone and treated the intermediates **4** or **5** with potassium *t*-butoxide in refluxing toluene to obtain the 2,3-dihydro-2-(2-oxopropylidene)- and 2,3-dihydro-2-(2-oxobutylidene)-4(1*H*)-pyrimidine ribofuranosides **7** and **8** in good yield (Scheme 1). In the case of phenacylbromide, alkylation of 2-thiouracil and ribosylation of the intermediate **3** under Vorbrüggen conditions [7] gave compound **6**, identical to that obtained from an alkylation of nucleoside **1**. This transformation was not possible using the pyrimidinone **2** since glycosylation under identical conditions did not yield the nucleoside product **4**. Also in contrast to **3**, compound **2** did not extrude sulfur when heated in DMF, consistent with similar results in the literature [8].

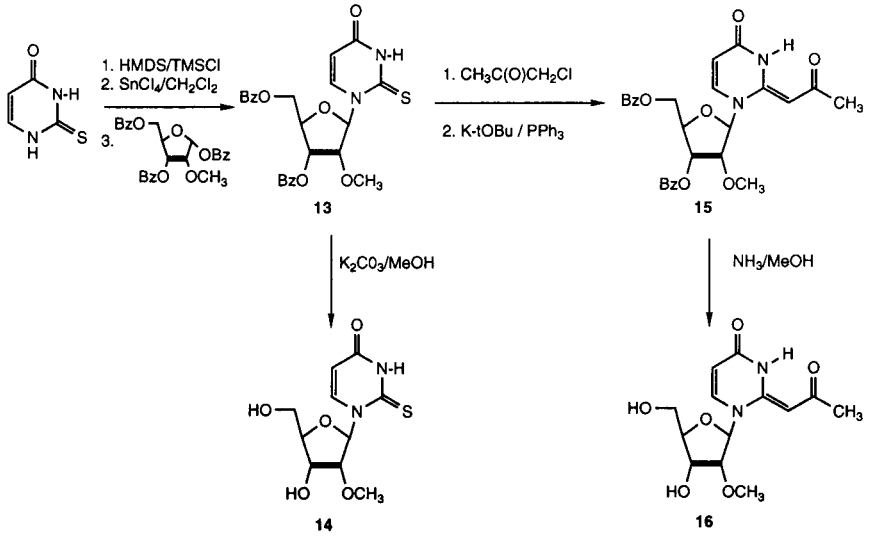
Removal of the benzoyl groups of nucleosides **7** or **8** using sodium methoxide in methanol unexpectedly gave the rearranged 2-methyl-1-(β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**12**). This product undoubtedly occurs *via* a retro-aldol reaction initiated by an attack of methoxide anion on the keto carbon of the tether. A milder condition for removal of the benzoyl groups using methanolic ammonia at room temperature gave the desired nucleoside analogs **9** and **10** in moderate yields, in addition to traces of the retro-aldol product **12**. Compound **12** was synthesized directly, by a glycosylation of 2-methyl-4(1*H*)-pyrimidinone [9] with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose followed by deprotection with sodium methoxide (Scheme 1). Synthesis of the corresponding 2′-*O*-methyl-2-thiopyrimidine ribofuranoside **13** was accomplished using the glycosylation method of Imbach [10] (Scheme 2). An alkylation of the thiocarbonyl group of **13** followed by an Eschenmoser sulfur extrusion gave the 2′-*O*-methyl nucleoside analog **15**. In contrast to the ribofuranosides **7** and **8**, the 2′-*O*-methyl ribofuranoside **15** was easily deprotected using methanolic ammonia or sodium methoxide to yield **16** without the aforementioned rearrangement.

At this juncture, we reasoned that the sulfur extrusion approach was limited by the unavailability of the 2′-fluoro-, 2′-methoxy- and 2′-deoxy-2-thiopyrimidines that we needed for future syntheses. Additionally, the bifunctionalized halomethyl ketones that we envisaged for conjugation to reactive moieties would not be stable under extrusion conditions. Development of a milder and more gener-

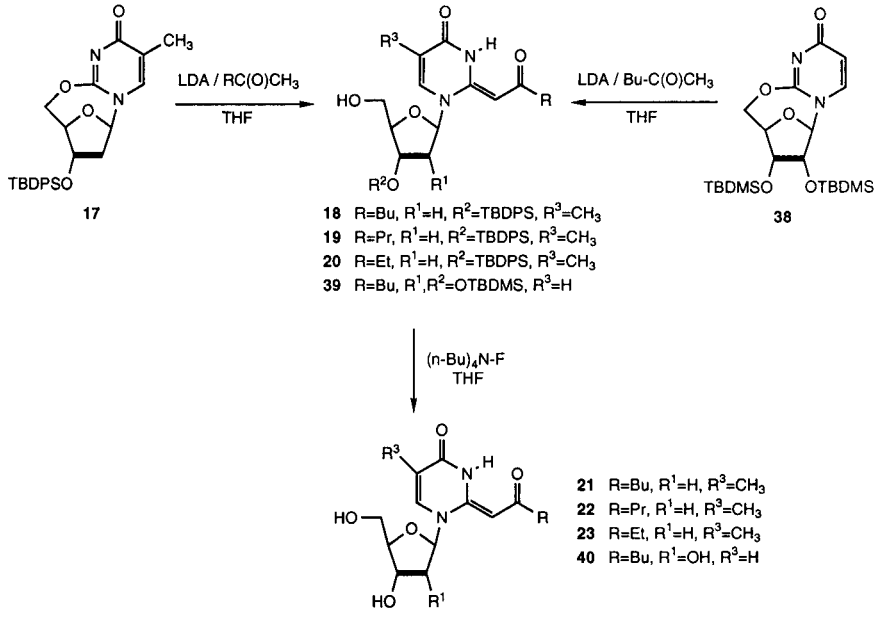
Scheme 1



Scheme 2

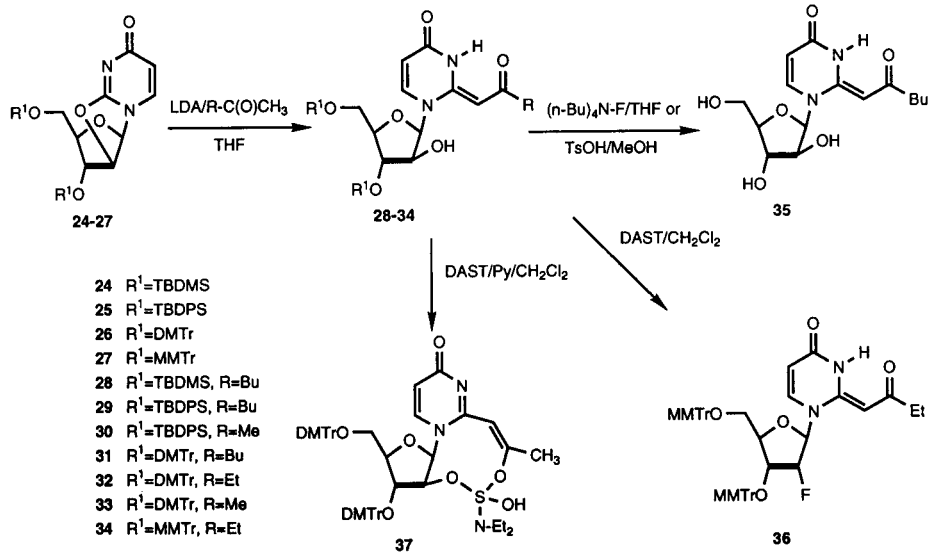


Scheme 3



al method for the introduction of β-ketoalkyl functionalities at the C-2 carbon of pyrimidines was required. Thus, we found that the direct introduction of a β-ketoalkyl group could be effected by the addition of lithium enolates of methylketones to anhydronucleosides protected with silyl or trityl groups (Scheme 3 and 4). Typically, a reaction at room temperature of 3′-*O*-*t*-butyldiphenylsilyl-2,5′-anhydrothymidine (**17**), with enolates of 2-hexanone, 2-pentanone or methylethyl ketone in THF gave the corresponding 2,3-dihydro-5-methyl-2-(2-oxoalkylidene)-1-(2-deoxy-3-*O*-*t*-butyldiphenylsilyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinones **18-20** in good yields. Following deprotection, nucleosides **21-23** were isolated as yellowish oils or foams with characteristic uv maxima near 320 nm. Further, the scope of this reaction was extended to other anhydronucleoside substrates. For example, 2,2′-anhydrouridine [11] protected with *t*-butyldimethylsilyl (TBDMS), *t*-butyldiphenylsilyl (TBDPS), dimethoxytrityl (DMTr) or monomethoxytrityl (MMTr) groups, **24-27**, was reacted with enolates under similar conditions (Scheme 4). The resulting 2,3-dihydro-2-(2-oxoalkylidene)-4(1*H*)-pyrimidinone nucleosides **28-34** were isolated in moderate to good yields. Following deprotection, the corresponding arabino nucleoside **35** was obtained. Additionally, the 2′,3′-di-*O*-TBDMS-2,5′-anhydrouridine [12] (**38**) was reacted with the enolate of 2-hexanone to give the intermediate **39** which on treatment with

Scheme 4



tetrabutylammonium fluoride afforded riboside **40** (Scheme 3). The ^{1}H nmr spectra of these compounds contain characteristic signals for the aglycone near 14 ppm, indicative of pyrimidines with imine/enol protons bound by N-3 and keto oxygen [13]. Further, two tautomeric structures may be drawn which account for the vinylic character (*ca* 5 ppm) of the alpha proton of the tether (Figure 1).

The protected intermediates **28-34** were reacted with diethylaminosulfur trifluoride (DAST) in dichloromethane in order to obtain the corresponding 2′-fluoro-2′-deoxyribofuranosides (Scheme 4). From these reactions, only the 2′-fluoro-2′-deoxyribofuranoside **36** could be isolated, albeit in low yield. All other reactions yielded intractable mixtures from which neither starting materials nor products could be isolated. Fluorination experiments using DAST which included pyridine as a co-solvent to prevent cleavage of the dimethoxytrityl groups of **33** yielded only an "anhydro" DAST adduct, tentatively assigned the structure **37**. An attempt to derivatize **31** with triflic anhydride for subsequent treatment with fluoride did not yield a triflate product and 85% of the starting material was recovered.

As mentioned previously, the chemistry of C-2 substituted pyrimidine nucleosides has not been thoroughly investigated. We believe all the compounds reported here are novel structures, with unique hydrogen bonding characteristics. The enol nature of these pyrimidines offers an N-3 imine proton and a 4-keto group as hydrogen bond donor and acceptor, respectively. The hydrogen bonding scheme we envision would involve a hydrogen bond from the 4-keto to the N-6 amino group of adenine. Second, the N-3 imino proton would exist in a bifurcated bond to the keto oxygen of the tether and to N-1 of adenine. Thus, one would expect that the strength of this Py-A hybrid pair would be weaker than a normal T-A pair [14]. Also, the bulk of tethers at pyrimidine C-2 might interfere with the approach of these nucleotides to their complement during hybridization. The incorporation of these nucleoside analogs into oligonucleotides and the study of their hybridization properties is in progress. The results of these and other synthetic efforts will be reported elsewhere.

EXPERIMENTAL

Melting points were measured on a Thomas Hoover capillary melting point apparatus and are uncorrected. The ^{1}H nmr spectra were recorded on a Varian Gemini 200 or a Varian Unity 400 spectrometer using a δ scale with tetramethylsilane as an internal standard. Rotary evaporations were carried out *in vacuo* at 35-45° using the combination of a vacuum pump and vacuum controller. The uv spectra were recorded on a Hewlett Packard 8452A Diode Array spectrophotometer. Thin layer chromatography was performed on Kieselgel 60 F-254 glass plates from E. Merck and compounds were visualized with uv light and/or sulfuric acid-methanol spray followed by charring. Flash chromatography was performed on silica gel (Baker 40 μm) according to the procedure of Still [15]. Drying of materials was achieved at atmospheric pressure and 100° or in a drying oven *in vacuo* at 40-60°. Elemental analyses were performed by Quantitative Technologies, Bound Brook, N.J. All reactions were performed under an argon atmosphere. Dry solvents and reagents were purchased from Aldrich unless otherwise noted.

1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-2-thio-4(1*H*,3*H*)-pyrimidinone (**1**).

A suspension of 2-thiouracil (0.58 g, 4.5 mmoles) and trimethylsilylchloride (0.5 ml) in hexamethyldisilazane (20 ml) was treated with ammonium sulfate (50 mg) and refluxed overnight. The clear greenish solution was evaporated with the exclusion of moisture. A solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose (2.52 g, 5

mmoles) in 1,2-dichloroethane (50 ml) was added and gave a clear solution after a few minutes. Stannic chloride (0.70 ml, 6 mmoles) was added to give a mixture that became momentarily turbid and then clear thereafter. After 1 hour the mixture was poured into a rapidly stirred mixture of saturated sodium bicarbonate (150 ml) and dichloromethane (300 ml). The organic layer was dried over sodium sulfate and evaporated to yield an amorphous residue which was purified by flash column chromatography (5 x 15 cm, chloroform/ethyl acetate, 6:1) to give a colorless foam, 2.23 g (88%). An analytical sample was obtained by crystallization from ethanol, mp 104° (lit [16] 105°); ^{1}H nmr (deuteriochloroform): δ 10.10 (s, 1H), 7.2-8.2 (m, 17H), 5.80 (m, 3H), 4.70 (m, 3H).

Anal. Calcd. for $C_{30}H_{24}N_2O_8S$ (572.57): C, 62.93; H, 4.22; N, 4.89. Found: C, 62.96; H, 4.12; N, 4.79.

2-(Acetonyl)thio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**4**).

A solution of **1** (1.073 g, 1.3 mmoles) and triethylamine (0.21 ml, 1.5 mmoles) in dichloromethane (10 ml) was treated with chloroacetone (0.153 ml, 1.5 mmoles). The reaction mixture was stirred at room temperature for 1 hour and then diluted with dichloromethane (30 ml). The solution was washed with water (20 ml), dried over sodium sulfate and evaporated to give a yellowish foam. The crude product is homogeneous by tlc and was used without further purification, 0.82 g (99%); ^{1}H nmr (deuteriochloroform): δ 8.15 (d, 2H), 8.05 (d, 2H), 7.95 (d, 2H), 7.70-7.50 (m, 10H), 6.45 (d, 1H), 5.90 (m, 2H), 5.70 (t, 1H), 4.90 (d, 1H), 4.75 (m, 2H), 4.25 (d, 1H), 4.07 (d, 1H), 2.00 (s, 3H).

2-(2-Oxobutyl)thio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**5**).

A solution of **1** (1.073 g, 1.3 mmoles) and triethylamine (0.21 ml, 1.5 mmoles) in dichloromethane (10 ml) was treated with 1-bromo-2-butanone (0.153 ml, 1.5 mmoles). The reaction mixture was stirred at room temperature for 1 hour and then was diluted with dichloromethane (30 ml). The solution was washed with water (20 ml), dried over sodium sulfate and evaporated to give a yellowish foam. The crude product is homogeneous by tlc and was used without further purification, 0.82 g (99%); ^{1}H nmr (deuteriochloroform): δ 8.15 (d, 2H), 8.05 (d, 2H), 7.95 (d, 2H), 7.70-7.50 (m, 10H), 6.45 (d, 1H), 5.90 (m, 2H), 5.70 (t, 1H), 4.90 (d, 1H), 4.75 (m, 2H), 4.25 (d, 1H), 4.07 (d, 1H), 2.65 (m, 2H), 1.10 (t, 3H).

2-(Phenacyl)thio-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**6**) [6].

Method A.

A solution of 2-(2-phenacyl)thio-1-4(1*H*)-pyrimidinone (**3**, 1.23 g, 5 mmoles), ammonium sulfate (50 mg) and trimethylsilyl chloride (1 ml) were refluxed in hexamethyldisilazane (25 ml) overnight. The yellow solution was evaporated and the oily residue was added to a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose (2.52 g, 5 mmoles) in 1,2-dichloroethane (50 ml). Stannic chloride (0.70 ml, 6 mmoles) was added in one portion and the solution was stirred for 30 minutes. The mixture was quenched with saturated sodium bicarbonate solution (20 ml) and the mixture extracted with chloroform (2 x 100 ml). The combined organic layers were dried over magnesium sulfate and evaporated to dryness. The residue was purified by flash column chromatography (5 x 15 cm, chloroform/ethyl acetate, 3:1) to give 1.58 g (45%) of a colorless foam.

Method B.

A solution of **1** (1.073 g, 1.3 mmoles) and triethylamine (0.21 ml, 1.5 mmoles) in dichloromethane (10 ml) was treated with phenacylbromide (0.153 ml, 1.5 mmoles). The mixture was stirred at room temperature for 1 hour and then diluted with dichloromethane (30 ml) and washed with water (20 ml). The organic layer was dried over sodium sulfate and evaporated to give a yellowish foam. The crude product is homogeneous by tlc, 0.82 g (99%); ^{1}H nmr (deuteriochloroform): δ 8.15-7.90 (m, 8H), 7.70-7.30 (m, 14H), 6.55 (dd, 1H), 5.90 (m, 2H), 5.70 (dd, 1H), 5.00 (d, 1H), 5.50 (d, 1H), 4.70-4.90 (m, 3H). These materials were used without further purification.

2,3-Dihydro-2-(2-oxopropylidene)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**7**).

Triphenylphosphine (7.87 g, 30 mmoles) and potassium *t*-butoxide (1 ml of a 1*M* solution in tetrahydrofuran, Aldrich) were added to a solution of **4** (6.29 g, 10 mmoles) in toluene (100 ml) and the mixture was refluxed for 18 hours. Upon cooling, the solution was evaporated and the residue was extracted with hot hexanes (3 x 100 ml) to remove triphenylphosphine and triphenylphosphine sulfide. The residue was purified by flash column chromatography (5 x 15 cm, chloroform/ethyl acetate, 7:3) to give 4.10 g (45%) of a colorless foam; ^{1}H nmr (deuteriochloroform): δ 13.80 (s, 1H), 8.10 (d, 2H), 8.00 (d, 2H), 7.90 (d, 2H), 7.70-7.40 (m, 10H), 5.90 (d, 1H), 5.80 (m, 2H), 5.50 (d, 1H), 5.00 (s, 1H), 4.90 (d, 1H), 4.80 (s, 1H), 4.70 (d, 1H), 2.00 (s, 3H).

Anal. Calcd. for $C_{33}H_{26}N_2O_9$ (596.6): C, 66.43; H, 4.73; N, 4.70. Found: C, 66.25; H, 4.66; N, 4.53.

2,3-Dihydro-2-(2-oxobutylidene)-1-(2',3',5'-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**8**).

Triphenylphosphine (1.05 g, 4 mmoles) and potassium *t*-butoxide (0.1 ml of a 1 *N* solution in tetrahydrofuran, Aldrich) was added to a solution of **5** (0.63 g, 1 mmole) in toluene (20 ml). The resultant yellow solution was refluxed for 3 hours. This reaction was worked up as for compound **7**. Materials were purified by flash column chromatography (3 x 15 cm, chloroform/ethyl acetate, 4:1) to give 0.44 g (74%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 13.80 (s, 1H), 8.10 (d, 2H), 8.00 (d, 2H), 7.95 (d, 2H), 7.70-7.40 (m, 10H), 5.90 (d, 1H), 5.80 (m, 2H), 5.50 (d, 1H), 5.00 (s, 1H), 4.90 (d, 1H), 4.80 (s, 1H), 4.70 (d, 1H), 2.25 (m, 2H), 1.05 (t, 3H).

Anal. Calcd. for $C_{34}H_{30}N_2O_9$ (610.0): C, 66.88; H, 4.96; N, 4.59. Found: C, 66.63; H, 4.90; N, 4.48.

2,3-Dihydro-2-(2-oxopropylidene)-1-(β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**9**).

A solution of **7** (2.85 g, 4.77 mmoles) in methanolic ammonia (60 ml) was stirred overnight at room temperature. The solution was evaporated to give an oily residue which was repeatedly triturated with dichloromethane (4 x 40 ml). The residue was purified by flash column chromatography (3 x 15 cm, chloroform/methanol, 2:1) to give 0.68 g (49%) of a yellowish, crystalline solid, mp 165°; ^{1}H nmr (deuteriochloroform): δ 13.90 (s, 1H), 8.10 (d, 1H), 5.65 (m, 2H), 5.35 (2, 1H), 5.15 (m, 3H), 4.00 (m, 3H), 3.70 (m, 1H), 3.40 (m, 1H), 2.30 (s, 3H).

Anal. Calcd. for $C_{12}H_{16}N_2O_6$ (284.3): C, 50.70; H, 5.67; N, 9.85. Found: C, 50.93; H, 5.68; N, 9.92.

2,3-Dihydro-2-(2-oxobutylidene)-1-(β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**10**).

A solution of **8** (1.22 g, 2.00 mmoles) in methanolic ammonia (30 ml) was stirred overnight at room temperature. The solution was evaporated to give an oily residue which was repeatedly triturated with dichloromethane (4 x 20 ml). The residue was purified by flash column chromatography (2 x 15 cm, chloroform/-methanol, 3:1) to give 0.33 g (55%) of a yellowish, crystalline solid, mp 156°; ^{1}H nmr (deuteriochloroform): δ 13.90 (s, 1H), 8.10 (d, 1H), 5.65 (m, 2H), 5.35 (2, 1H), 5.15 (m, 3H), 4.00 (m, 3H), 3.70 (m, 1H), 3.40 (m, 1H), 2.30 (q, 2H), 1.00 (t, 3H).

Anal. Calcd. for $C_{13}H_{18}N_2O_6$ (298.3): C, 52.35; H, 6.08; N, 9.39. Found: C, 52.49; H, 6.05; N. 9.54.

2-Methyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**11**).

A mixture of 2-methyl-4(1*H*)-pyrimidinone (4.42 g, 40 mmoles), ammonium sulfate (50 mg), trimethylsilyl chloride (0.5 ml) and hexamethyldisilazane (80 ml) was refluxed overnight. After cooling, the clear solution was evaporated and the amorphous residue was added to a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribose (20.18 g, 40 mmoles) in 1,2-dichloroethane (200 ml). Trimethylsilyl triflate (9.78 g, 44 mmoles) was added slowly and the clear reaction mixture was stirred at room temperature for 2 hours. The solution was poured into a rapidly stirred solution of sodium bicarbonate (300 ml) and the mixture was extracted with chloroform (4 x 150 ml). The combined organic extracts were dried over sodium sulfate and evaporated to give a colorless foam, 21.7 g (98%). The crude product was homogeneous by tlc and was used without further purification; ^{1}H nmr (deuteriochloroform): δ 8.10 (s, 1H), 8.05 (d, 1H), 7.95 (m, 4H), 7.70-7.35 (m, 10H), 6.15 (d, 1H), 6.00 (d, 1H), 5.80 (m, 1H), 5.65 (dd, 1H), 4.85 (dd, 1H), 4.80-4.65 (m, 2H), 2.60 (s, 3H); N-1 substitution on the heterocycle was proven by a ^{1}H NOESY spectrum of **12**.

2-Methyl-1-(β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**12**).

Method A.

A solution of **7** (13.86 g, 23 mmoles) in methanol (150 ml) was treated with sodium methoxide (2.3 mmoles)) and the clear solution was stirred overnight at room temperature. Evaporation of the solvent gave a residue which was partitioned between water (100 ml) and dichloromethane (400 ml). The aqueous layer was neutralized with Dowex 50 (H^+ form) and evaporated. The oily residue which resulted was crystallized from ethanol/acetone to give 2.37 g (42%) of a colorless, crystalline solid.

Method B.

A solution of **11** (3.90 g, 6.53 mmoles) in methanol (100 ml) was treated with sodium methoxide (7.84 mmoles) and stirred at room temperature overnight. Evaporation of the solvent gave a residue which was partitioned between water (120 ml) and diethylether (300 ml). The aqueous layer was neutralized with 10% acetic acid and evaporated. Flash column chromatography (4 x 15 cm, chloroform/methanol, 2:1) yielded 0.64 g (40%) of a slightly yellowish solid, mp 168°; ^{1}H nmr (DMSO-d_6): δ 8.05 (d, 1H), 6.00 (d, 1H), 5.65 (m, 2H), 5.30 (d, 1H), 5.20 (t, 1H), 4.00 (m, 3H), 3.60 (m, 2H), 2.45 (s, 3H); N-1 substitution on the heterocyclic system was proven by a ^{1}H NOESY experiment.

Anal. Calcd. for $C_{10}H_{14}N_2O_5$ (242.2): C, 49.59; H, 5.83; N, 11.57. Found: C, 49.16; H, 6.20; N, 11.35.

1-(3,5-Di-*O*-benzoyl-2-*O*-methyl-β-D-ribofuranosyl)-2-thio-4-(1*H*,3*H*)-pyrimidinone (**13**).

A mixture of 2-thiouracil (1.28 g, 10 mmoles), dry ammonium sulfate (50 mg), trimethylsilyl chloride (0.5 ml) and hexamethyldisilazane (20 ml) was refluxed overnight. The clear, greenish solution was evaporated with the exclusion of moisture. A solution of 2-*O*-methyl-1,3,5-tri-*O*-benzoyl-α-D-ribose (4.76 g, 10 mmoles) in acetonitrile (50 ml) was added in one portion followed by stannic chloride (2.60 g, 10 mmoles). After 3 hours, the solution was poured into a rapidly stirred mixture of saturated sodium bicarbonate (200 ml) and dichloromethane (400 ml). The organic layer was separated, dried over sodium sulfate and evaporated to dryness. The resulting amorphous residue was crystallized from hot ethanol to give colorless needles, 1.89 g (39%), mp 159°; ^{1}H nmr (deuteriochloroform): δ 10.30 (s, 1H), 8.10 (m, 2H), 8.05 (m, 2H), 7.80 (d, 1H), 7.50 (m, 6H), 6.70 (s, 1H), 5.70 (dd, 1H), 5.25 (m, 1H), 4.90 (d, 1H), 4.80 (d, 1H), 4.65 (d, 1H), 4.30 (d, 1H), 3.60 (s, 3H).

Anal. Calcd. for $C_{24}H_{22}N_2O_7S$ (482.5): C, 59.74; H, 4.60; N, 5.81; S, 6.65. Found: C, 59.36; H, 4.63; N, 5.61; S, 6.31.

1-(2-*O*-Methyl-β-D-ribofuranosyl)-2-thio-4(1*H*,3*H*)-pyrimidinone (**14**).

A solution of **13** (0.483 g, 1 mmole) in anhydrous methanol (15 ml) was treated with potassium carbonate (0.15 g, 1.1 mmoles) and the mixture was stirred overnight at room temperature. The mixture was evaporated and the residue was partitioned between water (10 ml) and ether (30 ml). The aqueous layer was neutralized with 10% acetic acid and evaporated. The residue was crystallized to give 0.194 g (59%) of colorless needles in two crops, mp 248° dec. This material proved to be somewhat unstable and did not fit elemental analysis; ^{1}H nmr (DMSO-d_6): δ 12.35 (s, 1H), 7.65 (d, 1H), 7.00 (d, 1H), 5.45 (d, 1H), 5.00 (m, 3H), 4.00 (t, 1H), 3.70 (m, 1H), 3.65 (d, 1H), 3.50 (m, 2H), 3.45 (s, 3H). The β-configuration was established by a ^{1}H NOESY experiment.

2,3-Dihydro-2-(2-oxopropylidene)-1-(3,5-di-*O*-benzoyl-2-*O*-methyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**15**).

Chloroacetone (0.35 ml, 4.35 mmoles) was added to a solution of **13** in dichloromethane (20 ml) and triethylamine (0.81 ml, 5.80 mmoles) at room temperature. After 16 hours water (20 ml) was added and the organic layer was shaken repeatedly with water. The organic layer was dried over sodium sulfate and evaporated to dryness. The yellowish crude product was dissolved in toluene (20 ml) and immediately treated with triphenylphosphine and potassium *t*-butoxide (0.29 ml of a 1 *N* solution in tetrahydrofuran, Aldrich). The mixture was stirred at 125° overnight. Evaporation of the solvent followed by purification of the residue by flash column chromatography (5 x 15 cm; chloroform/ethyl acetate, 2:1) yielded 1.26 g (80%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 13.80 (s, 1H), 8.10 (d, 2H), 8.05 (d, 2H), 7.70-7.40 (m, 7H), 5.60-5.40 (m, 3H), 4.95 (s, 1H), 4.80 (d, 1H), 4.70 (m, 2H), 4.10 (s, 1H), 3.50 (s, 3H), 2.20 (s, 3H).

Anal. Calcd. for $C_{27}H_{26}N_2O_8$ (506.5): C, 64.03; H, 5.17; N, 5.53. Found: C, 64.50; H, 5.17; N, 5.14.

2,3-Dihydro-2-(2-oxopropylidene)-1-(2-*O*-methyl)-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**16**).

A solution of **15** (0.51 g, 1 mmole) and methanolic ammonia (30 ml, saturated at 0°) in a pressure bottle was stirred overnight at room temperature. Evaporation of the solution gave a yellowish residue which was repeatedly triturated with ether (4 x 30 ml). The residue was further purified by flash column chromatography (2 x 15 cm, chloroform/methanol, 85:15) to give 0.41 g

(59%) of yellowish crystals, mp 163°; ^{1}H nmr (deuteriochloroform): δ 13.95 (s, 1H), 8.05 (d, 1H), 5.75 (d, 1H), 5.50 (s, 1H), 5.30 (d, 1H), 5.25 (t, 1H), 5.15 (s, 1H), 4.15 (m, 1H), 3.90 (m, 1H), 3.85 (m, 1H), 3.70 (d, 1H), 3.60 (d, 1H), 2.05 (s, 3H).

Anal. Calcd. for $C_{13}H_{18}N_2O_6$ (298.3): C, 52.35; H, 6.08; N, 9.39. Found: C, 52.54; H, 6.08; N, 9.55.

2,5'-Anhydro-3'-*O*-*t*-butyldiphenylsilylthymidine (**17**).

A solution of 2,5'-anhydrothymidine (2.24 g, 10 mmoles) [17] and imidazole (2.04 g, 30 mmoles) in pyridine (100 ml) was treated with *t*-butyldiphenylsilyl chloride (2.37 ml, 15 mmoles) and stirred overnight at room temperature. The mixture was evaporated to dryness and the residue was partitioned between chloroform (100 ml) and water (50 ml). The organic layer was washed with additional water, dried over sodium sulfate and evaporated. The resulting oily residue was purified by flash column chromatography (5 x 15 cm, chloroform/methanol, 9:1) to yield 3.47 g (75%) of the title compound. An analytical sample was obtained by crystallization from methanol, mp 232°; ^{1}H nmr (deuteriochloroform): δ 7.65 (m, 4H), 7.45 (m, 6H), 7.10 (s, 1H), 5.65 (d, 1H), 4.70 (d, 1H), 4.10 (s, 1H), 3.85 (dd, 2H), 2.55 (dd, 1H), 2.45 (dd, 1H), 1.95 (s, 3H), 1.10 (s, 9H).

Anal. Calcd. for $C_{20}H_{30}N_2O_4Si$ (462.86): C, 67.50; H, 6.54; N, 6.06. Found: C, 67.29; H, 6.58; N, 6.02.

2,3-Dihydro-5-methyl-2-(2-oxohexylidene)-1-(2-deoxy-3-*O*-*t*-butyldiphenylsilyl-β-D-*erythro*-pentofuranosyl)-4(1*H*)-pyrimidinone (**18**).

A solution of 2-hexanone (1.50 ml, 12 mmoles) in tetrahydrofuran (60 ml) was cooled to -70°. Lithium diisopropylamide (6 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **17** (1.74 g, 3.76 mmoles) was added to give an immediate yellow solution. Tlc after 35 minutes indicated complete reaction. The reaction was quenched with the addition of water (10 ml) and the mixture was neutralized carefully with 1 *N* hydrochloric acid. The mixture was partitioned between ether (150 ml) and water (50 ml); the organic layer was dried over sodium sulfate and evaporated to dryness. The resulting yellow foam was purified by flash column chromatography (5 x 15 cm; ethyl acetate) to yield 1.47 g (69%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 14.10 (s, 1H), 7.65 (m, 4H), 7.45 (m, 6H), 5.80 (t, 1H), 4.80 (s, 1H), 4.45 (m, 3H), 4.05 (t, 1H), 3.75 (d, 1H), 3.40 (d, 1H), 2.35 (m, 3H), 2.00 (m, 1H), 1.85 (m, 4H), 1.60 (m, 2H), 1.35 (m, 2H), 1.10 (s, 9H), 0.90 (t, 3H).

Anal. Calcd. for $C_{32}H_{42}N_2O_5Si$ (562.8): C, 68.30; H, 7.52; N, 4.98. Found: C, 68.08; H, 7.64; N, 4.78.

2,3-Dihydro-5-methyl-2-(2-oxopentylidene)-1-(2-deoxy-3-*O*-*t*-butyldiphenylsilyl-β-D-*erythro*-pentofuranosyl)-4(1*H*)-pyrimidinone (**19**).

A solution of 2-pentanone (0.64 ml, 6 mmoles) in tetrahydrofuran (40 ml) was cooled to -70°. Lithium diisopropylamide (3 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **17** (0.93 g, 2 mmoles) was added to give an immediate yellow color. Tlc after 35 minutes indicated complete reaction. The reaction was worked up as for compound **18**. Flash column chromatography (3 x 15 cm, ethyl acetate) yielded 0.79 g (71%) of a slightly yellowish foam; ^{1}H nmr (deuteriochloroform): δ 14.05 (s, 1H), 7.65 (m, 4H), 7.45 (m, 7H), 5.75 (t, 1H), 4.80 (s, 1H), 4.45 (m, 1H), 4.05 (m, 1H), 3.70 (dd, 1H), 3.40 (dd, 1H), 2.30 (m, 3H), 2.00 (m, 1H), 1.95 (d, 1H), 1.80 (s, 3H), 1.60 (m, 2H), 1.05 (s, 9H), 0.95 (t, 3H).

Anal. Calcd. for $C_{31}H_{40}N_2O_5Si$ (548.8): C, 67.85; H, 7.35; N, 5.10. Found: C, 67.56; H, 7.45; N, 5.00.

2,3-Dihydro-5-methyl-2-(2-oxobutylidene)-1-(2-deoxy-3-*O*-*t*-butyldiphenylsilyl-β-D-*erythro*-pentofuranosyl)-4(1*H*)-pyrimidinone (**20**).

A solution of 2-butanone (0.54 ml, 6 mmoles) in tetrahydrofuran (40 ml) was cooled to -70°. Lithium diisopropylamide (3 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **17** (0.93 g, 2 mmoles) was added and the solution gave an immediate yellow color. Tlc after 35 minutes indicated complete reaction. The reaction was worked up as for compound **18**. Flash column chromatography (3 x 15 cm; ethyl acetate) yielded 0.51 g (47%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 14.05 (s, 1H), 7.65 (m, 4H), 7.45 (m, 7H), 7.75 (t, 1H), 4.80 (s, 1H), 4.45 (m, 1H), 4.05 (m, 1H), 3.70 (dd, 1H), 3.40 (dd, 1H), 2.30 (m, 3H), 2.00 (m, 1H), 1.80 (s, 4H), 1.05 (m, 12H).

Anal. Calcd. for $C_{30}H_{38}N_2O_5Si$ (534.7): C, 67.38; H, 7.16; N, 5.24. Found: C, 67.31; H, 7.20; N, 5.17.

2,3-Dihydro-5-methyl-2-(2-oxohexylidene)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4(1*H*)-pyrimidinone (**21**).

A solution of **18** (0.75 g, 1.33 mmoles) in tetrahydrofuran (30 ml) was treated with tetrabutylammonium fluoride hydrate (0.39 g, 1.50 mmoles) and stirred at room temperature overnight. The mixture was evaporated and the resulting residue was purified by flash column chromatography (3 x 15 cm, ethyl acetate/methanol, 19:1) to yield 0.30 g (69%) of a colorless foam; ^{1}H nmr (DMSO-d_6): δ 14.25 (s, 1H), 7.95 (s, 1H), 5.80 (t, 1H), 5.30 (s, 1H), 5.20 (t, 1H), 5.10 (s, 1H), 4.25 (m, 1H), 3.80 (m, 1H), 3.65 (m, 1H), 3.55 (m, 1H), 2.25 (m, 3H), 2.20 (m, 1H), 1.80 (s, 3H), 1.50 (m, 2H), 1.30 (m, 2H), 0.85 (t, 3H).

Anal. Calcd. for $C_{16}H_{24}N_2O_5 \cdot 0.50H_2O$ (333.4): C, 57.64; H, 7.56; N, 8.40. Found: C, 57.64; H, 7.31; N, 8.31.

2,3-Dihydro-5-methyl-2-(2-oxopentylidene)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4(1*H*)-pyrimidinone (**22**).

A solution of **19** (0.65 g, 1.18 mmoles) in tetrahydrofuran (30 ml) was treated with tetrabutylammonium fluoride hydrate (0.56 g, 1.78 mmoles) and stirred at room temperature overnight. The mixture was evaporated to dryness and the resulting residue was purified by flash column chromatography (3 x 15 cm, ethyl acetate/methanol, 19:1) to yield 0.27 g (75%) of a colorless foam; ^{1}H nmr (DMSO-d_6): δ 14.25 (s, 1H), 7.95 (s, 1H), 5.80 (t, 1H), 5.30 (s, 1H), 5.15 (t, 1H), 5.05 (s, 1H), 4.25 (m, 1H), 3.80 (m, 1H), 3.60 (m, 2H), 2.2 (m, 4H), 1.80 (s, 3H), 1.50 (m, 2H), 1.55 (m, 2H), 0.85 (t, 3H).

Anal. Calcd. for $C_{15}H_{22}N_2O_5 \cdot 0.75H_2O$ (323.9): C, 55.63; H, 7.31; N, 8.63. Found: C, 55.53; H, 7.03; N, 8.44.

2,3-Dihydro-5-methyl-2-(2-oxobutylidene)-1-(2-deoxy-β-D-*erythro*-pentofuranosyl)-4(1*H*)-pyrimidinone (**23**).

A solution of **20** (0.40 g, 0.75 mmole) in tetrahydrofuran (20 ml) was treated with tetrabutylammonium fluoride hydrate (0.35 g, 1.13 mmoles) and stirred at room temperature overnight. The mixture was evaporated to dryness and the resulting residue was purified by flash column chromatography (3 x 15 cm, ethyl acetate/methanol, 19:1) to yield 0.16 g (72%) of a colorless foam; ^{1}H nmr (DMSO-d_6): δ 14.25 (s, 1H), 7.95 (s, 1H), 5.80 (t, 1H), 5.30 (s,

1H), 5.15 (t, 1H), 5.05 (s, 1H), 4.25 (m, 1H), 3.80 (m, 1H), 3.65 (dd, 1H), 3.60 (dd, 1H), 2.25 (m, 3H), 2.15 (m, 1H), 1.80 (s, 3H), 1.50 (m, 2H), 1.55 (m, 2H), 1.00 (t, 3H).

Anal. Calcd. for $C_{14}H_{20}N_2O_5 \cdot 0.5H_2O$ (305.3): C, 55.07; H, 6.93; N, 9.17. Found: C, 55.40; H, 6.99; N, 8.89.

2,2'-Anhydro-1-(3,5-di-*O*-*t*-butyldimethylsilyl-β-D-arabinofuranosyl)uracil (**24**).

A solution of 2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil (4.53 g, 20 mmoles) and imidazole (10.89 g, 0.16 mole) in pyridine (150 ml) was treated with *t*-butyldimethylsilyl chloride (12.1 g, 80 mmoles). The mixture was stirred at room temperature overnight and then evaporated to dryness to give a yellowish residue which was partitioned between chloroform (400 ml) and water (250 ml). The organic layer was separated, dried over sodium sulfate and evaporated. The resulting residue was purified by flash column chromatography (7 x 10 cm, chloroform/methanol, 19:1) to give 5.56 g (61%) of a colorless solid, mp 152°; ^{1}H nmr (deuteriochloroform): δ 7.35 (s, 1H), 6.13 (d, 1H), 6.07 (d, 1H), 5.10 (d, 1H), 4.60 (s, 1H), 4.15 (m, 1H), 3.57 (dd, 1H), 3.38 (dd, 1H), 0.92 (s, 9H), 0.85 (s, 9H).

Anal. Calcd. for $C_{21}H_{38}N_2O_5Si_2$ (454.7): C, 55.47; H; 8.42; N, 6.16. Found: C, 55.46; H, 8.48; N, 6.13.

2,2'-Anhydro-1-(3,5-di-*O*-*t*-butyldiphenylsilyl-β-D-arabinofuranosyl)uracil (**25**).

A solution of 2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil (4.53 g, 20 mmoles) and imidazole (10.89 g, 0.16 mole) in pyridine (100 ml) was treated with *t*-butyldiphenylsilyl chloride (20.5 ml, 80 mmoles). The mixture was stirred at room temperature overnight and then evaporated to dryness to give a yellowish foam which was partitioned between ethyl acetate (500 ml) and water (300 ml). The organic layer was separated, dried over sodium sulfate and evaporated. The resulting residue was purified by flash column chromatography (7 x 15 cm, ethyl acetate) to yield 11.9 g (85%) of a colorless foam; ^{1}H nmr (deuteriochloroform): δ 7.60 (d, 4H), 7.40 (m, 17H), 6.10 (d, 1H), 5.95 (d, 1H), 5.07 (d, 1H), 4.60 (s, 1H), 4.35 (t, 1H), 3.25 (dd, 1H), 3.15 (dd, 1H), 1.10 (s, 9H), 0.90 (s, 9H).

Anal. Calcd. for $C_{41}H_{46}N_2O_5Si_2$ (703.0): C, 70.05; H, 6.60; N, 3.98. Found: C, 69.89; H, 6.55; N, 3.97.

2,2'-Anhydro-1-(3,5-di-*O*-dimethoxytrityl-β-D-arabinofuranosyl)-uracil (**26**).

Dimethoxytrityl chloride (16.94 g, 50 mmoles) was added to a solution of 2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil (4.53 g, 20 mmoles) in pyridine (130 ml). The mixture was stirred at room temperature and then evaporated to dryness to give a yellowish residue which was partitioned between ethyl acetate (400 ml) and water (200 ml). The combined organic layers were dried over sodium sulfate and evaporated. The resulting residue was purified by flash column chromatography (7 x 15 cm, ethyl acetate) to yield 9.88 g (59%) of a colorless foam. This material was homogenous by tlc and was used without further purification; ^{1}H nmr (deuteriochloroform): δ 7.40 (d, 1H), 7.20 (m, 18H), 6.80 (m, 8H), 5.95 (d, 1H), 5.90 (d, 1H), 4.80 (d, 1H), 4.30 (s, 1H), 3.90 (d, 1H), 3.80 (s, 6H), 3.75 (s, 3H), 3.70 (s, 3H), 2.90 (dd, 1H), 2.77 (dd, 1H).

2,2-Anhydro-1-(3,5-di-*O*-monomethoxytrityl-β-D-arabinofuranosyl)uracil (**27**).

Monomethoxytrityl chloride (13.90 g, 45 mmoles) was added to a solution of 2,2'-anhydro-1-(β-D-arabinofuranosyl)uracil (4.53 g, 20 mmoles) in pyridine (130 ml). This mixture was stirred at 80° for 6 hours and then evaporated to yield a yellowish residue which was partitioned between ethyl acetate (400 ml) and water (200 ml). The combined organic layers were dried over sodium sulfate, filtered and evaporated. The resulting residue was purified by flash column chromatography (7 x 15 cm, ethyl acetate/-methanol, 19:1) to give 9.80 g (63%) of a colorless foam. This material was homogeneous by tlc and was used without further purification; ^{1}H nmr (deuteriochloroform): δ 7.40 (m, 4H), 7.30-7.10 (m, 21H), 7.15 (d, 2H), 6.80 (m, 4H), 5.95 (d, 1H), 5.85 (d, 1H), 4.80 (t, 1H), 4.35 (s, 1H), 3.90 (d, 1H), 3.80 (s, 3H), 3.75 (s, 3H), 2.85 (dd, 1H), 2.77 (dd, 1H).

2,3-Dihydro-2-(2-oxohexylidene)-1-(3,5-di-*O*-*t*-butyldimethylsilyl-β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**28**).

A solution of 2-hexanone (1.11 ml, 9 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (4.5 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **24** (1.36 g, 3 mmoles) was added and gave an immediate yellowish color. Tlc after 35 minutes indicated complete reaction. The reaction was quenched with the addition of water (10 ml) and then carefully neutralized with 1 *N* hydrochloric acid. The mixture was partitioned between ether (150 ml) and water (50 ml), the organic layer was separated and dried over magnesium sulfate. Evaporation of the solvent and purification by flash column chromatography (3 x 15 cm, hexanes/ethyl acetate 2:1) yielded 0.87 g (52%) of a yellowish oil; ^{1}H nmr (deuteriochloroform): δ 13.90 (s, 1H), 7.65 (d, 1H), 5.60 (m, 2H), 4.85 (s, 1H), 4.80 (s, 1H), 4.25 (s, 1H), 4.15-3.80 (m, 3H), 2.55 (d, 1H), 2.40 (t, 1H), 2.25 (t, 1H), 1.55 (m, 2H), 1.30 (m, 2H), 0.90 (m, 21H), 0.15 (m, 12H).

Anal. Calcd. for $C_{27}H_{50}N_2O_6Si_2$ (554.9): C, 58.44; H, 9.08; N, 5.05. Found: C, 57.98; H, 9.27; N, 5.13.

2,3-Dihydro-2-(2-oxohexylidene)-1-(3,5-di-*O*-*t*-butyldiphenylsilyl-β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**29**).

A solution of 2-hexanone (1.11 ml, 9 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (4.5 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **25** (2.11 g, 3 mmoles) was added and gave an immediate yellow color. Tlc after 35 minutes indicated complete reaction. The reaction was worked up as for compound **28**. Flash column chromatography (3 x 15 cm, hexanes/ethyl acetate, 2:1) yielded 1.14 g (47%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 13.90 (s, 1H), 7.70-7.20 (m, 21H), 5.75 (s, 1H), 5.50 (dd, 1H), 4.30 (s, 1H), 4.10 (m, 2H), 4.05 (s, 1H), 3.70 (dd, 1H), 3.20 (dd, 1H), 2.30 (t, 2H), 1.60 (m, 2H), 1.35 (m, 2H), 1.10 (s, 9H), 0.90 (m, 12H).

Anal. Calcd. for $C_{47}H_{58}N_2O_6Si_2$ (803.2): C, 70.29; H, 7.28; N, 3.49. Found: C, 70.15; H, 7.36; N, 3.39.

2,3-Dihydro-2-(2-oxopropylidene)-1-(3,5-di-*O*-*t*-butyldiphenylsilyl-β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**30**).

A solution of acetone (1.32 ml, 18 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (9 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **25** (4.22 g, 6 mmoles) was added and gave an immediate yellow color. Tlc after 35 minutes indicated complete reaction. This reaction was worked up as for compound **28**. Flash column chromatography (7 x 15 cm, hexanes/-

ethyl acetate, 2:1) yielded 2.06 g (45%) of a colorless foam; ^{1}H nmr (deuteriochloroform): δ 13.80 (s, 1H), 7.70-7.20 (m, 21H), 5.75 (s, 1H), 5.55 (dd, 1H), 4.80 (s, 1H), 4.35 (s, 1H), 4.25 (m, 1H), 4.15 (m, 1H), 4.05 (s, 1H), 3.70 (dd, 1H), 3.25 (dd, 1H), 2.10 (s, 3H), 1.10 (s, 9H), 1.00 (s, 9H).

Anal. Calcd. for $C_{44}H_{52}N_2O_6Si_2$ (761.1): C, 69.44; H, 6.89; N, 3.68. Found: C, 68.95; H, 7.05; N, 3.57.

2,3-Dihydro-2-(2-oxohexylidene)-1-(3,5-di-*O*-dimethoxytrityl-β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**31**).

A solution of 2-hexanone (1.48 ml, 12 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (6 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **26** (3.20 g, 3.85 mmoles) was added and gave an immediate yellow color. Tlc after 35 minutes indicated complete reaction. This reaction was worked up as for compound **33**. Flash column chromatography (5 x 15 cm, hexanes/ethyl acetate, 1:2) yielded 1.60 g (45%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 13.85 (s, 1H), 7.60 (s, 1H), 7.25 (m, 18H), 6.80 (m, 8H), 5.60 (m, 2H), 4.60 (s, 1H), 4.15 (t, 1H), 4.00 (d, 1H), 3.80 (s, 12H), 3.70 (d, 1H), 3.50 (d, 1H), 3.40 (d, 1H), 2.25 (m, 2H), 1.60 (m, 2H), 1.25 (m, 2H), 0.90 (t, 3H).

Anal. Calcd. for $C_{57}H_{58}N_2O_{10}$ (931.0): C, 73.53; H, 6.28; N, 3.01. Found: C, 73.15; H, 6.31; N, 2.96.

2,3-Dihydro-2-(2-oxobutylidene)-1-(3,5-di-*O*-dimethoxytrityl-β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**32**).

A solution of 2-butanone (1.10 ml, 12 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (6 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **26** (3.32 g, 4 mmoles) was added and gave an immediate yellow color. Tlc after 40 minutes indicated complete reaction. This reaction was worked up as for compound **28**. Flash column chromatography (5 x 15 cm, hexanes/ethyl acetate, 1:2) yielded 1.42 g (39%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 13.80 (s, 1H), 7.60 (s, 1H), 7.25 (m, 18H), 6.80 (m, 8H), 5.60 (m, 2H), 4.60 (s, 1H), 4.10 (m, 2H), 3.80 (m, 12H), 3.50 (d, 1H), 3.40 (m, 1H), 3.40 (d, 1H), 3.20 (d, 1H), 2.25 (m, 2H), 1.10 (t, 3H).

Anal. Calcd. for $C_{55}H_{54}N_2O_{10}$ (903.0): C, 73.15; H, 6.03; N, 3.10. Found: C, 72.94; H, 6.16; N, 3.03.

2,3-Dihydro-2-(2-oxopropylidene)-1-(3,5-di-*O*-dimethoxytrityl-β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**33**).

A solution of acetone (0.44 ml, 6 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (3 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **26** (1.60 g, 2 mmoles) was added and gave an immediate yellow color. Tlc after 40 minutes indicated complete reaction. This reaction was worked up as for compound **28**. Flash column chromatography (5 x 15 cm, hexanes/ethyl acetate, 1:2) yielded 1.42 g (28%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 13.80 (s, 1H), 7.60 (s, 1H), 7.40-7.15 (m, 18H), 6.80 (m, 8H), 5.60 (m, 2H), 4.60 (s, 1H), 4.10 (m, 1H), 4.00 (s, 1H), 3.80 (m, 12H), 3.50 (d, 1H), 3.40 (m, 1H), 3.20 (d, 1H), 2.05 (s, 3H).

Anal. Calcd. for $C_{54}H_{52}N_2O_{10}\cdot H_2O$ (907.0): C, 71.51; H, 6.00; N, 3.09. Found: C, 71.37; H, 6.00; N, 3.09.

2,3-Dihydro-2-(2-oxobutylidene)-1-(3,5-di-*O*-monomethoxytrityl-β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**34**).

A solution of 2-butanone (1.34 ml, 15 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (6 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes, then warmed to room temperature. Compound **27** (3.85 g, 5 mmoles) was added and gave an immediate yellow color. Tlc after 1 hour indicated complete reaction. This reaction was worked up as for compound **28**. Flash column chromatography (5 x 15 cm, hexanes/ethyl acetate, 1:2) yielded 1.32 g (34%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 13.80 (s, 1H), 7.60 (s, 1H), 7.25 (m, 24H), 6.80 (d, 4H), 5.60 (m, 2H), 4.10 (s, 1H), 4.00 (s, 1H), 3.80 (m, 10H), 3.45 (m, 2H), 3.20 (m, 1H), 2.25 (m, 2H), 1.10 (t, 3H).

Anal. Calcd. for $C_{53}H_{50}N_2O_8$ (843.0): C, 75.51; H, 5.98; N, 3.32. Found: C, 75.04; H, 6.07; N, 3.21.

2,3-Dihydro-2-(2-oxohexylidene)-1-(β-D-arabinofuranosyl)-4(1*H*)-pyrimidinone (**35**).

Method A.

A solution of **29** (1.43 g, 1.78 mmoles) or **28** (0.99 g, 1.78 mmoles) in tetrahydrofuran (30 ml) was treated with tetrabutylammonium fluoride hydrate (1.16 g, 4.45 mmoles) and the mixture stirred at room temperature for 1 hour. Evaporation of the solvents yielded a residue which was purified by flash column chromatography (3 x 15 cm, ethyl acetate/methanol, 19:1) to yield 295 mg (52%) of a colorless amorphous solid.

Method B.

Compound **31** (2.33 g, 2.5 mmoles) was treated with a solution of 2% toluenesulfonic acid in dichloromethane/methanol, 4:1 (100 ml) and stirred for 30 minutes at room temperature. The mixture was evaporated and the residue purified by flash column chromatography (4 x 15 cm, ethyl acetate/methanol, 19:1) to yield 0.32 g (39%) of a colorless amorphous solid; ^{1}H nmr (deuteriochloroform): δ 14.05 (s, 1H), 7.80 (d, 1H), 5.75 (s, 1H), 5.70 (m, 2H), 5.50 (d, 1H), 5.10 (s, 1H), 5.05 (t, 1H), 4.15 (s, 1H), 3.90 (m, 1H), 3.75 (m, 1H), 3.60 (m, 2H), 2.25 (m, 2H), 1.50 (m, 2H), 1.25 (m, 2H), 0.85 (t, 3H).

Anal. Calcd. for $C_{15}H_{22}N_2O_6$ (326.4): C, 55.21; H, 6.80; N, 8.58. Found: C, 55.53; H, 6.89; N, 8.22.

2,3-Dihydro-2-(2-oxobutylidene)-1-(2-deoxy-2-fluoro-3,5-di-*O*-monomethoxytrityl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**36**).

Diethylaminosulfur trifluoride (0.20 ml, 1.5 mmoles) was added to a solution of compound **34** (0.84 g, 1 mmole) in dichloromethane (10 ml). The yellowish solution was stirred at room temperature for 2 hours and then quenched with the addition of aqueous sodium bicarbonate (20 ml) and dichloromethane (50 ml). The organic layer was dried over sodium sulfate and evaporated to dryness. The residue which resulted was purified by flash column chromatography (3 x 15 cm, ethyl acetate/hexanes, 3:2) to yield 85 mg (10%) of a yellowish oil. This material gave an ^{1}H nmr consistent with the structure proposed but did not fit elemental analysis; ^{1}H nmr (deuteriochloroform): δ 13.70 (s, 1H), 7.65 (d, 1H), 7.00-7.50 (m, 24H), 6.80 (m, 4H), 5.45 (d, 1H), 5.00 (d, 1H), 4.60 (s, 1H), 4.25-4.00 (m, 2H), 3.85-3.65 (m, 7.5H), 3.60-3.40 (m, 2.5H), 2.35 (m, 2H), 1.10 (t, 3H).

DAST Adduct **37**.

Diethylaminosulfur trifluoride (0.13 ml, 1 mmole) was added to

a solution of **33** (0.44 g, 0.49 mmole) in dichloromethane (10 ml) and pyridine (2 ml). Tlc indicated complete conversion to a polar product after 1 hour. The reaction was quenched with aqueous sodium bicarbonate (20 ml) and extracted with dichloromethane (50 ml). The organic layer was dried over sodium sulfate and evaporated to dryness. Purification by flash column chromatography (3 x 15 cm, chloroform/methanol, 9:1) yielded 0.35 g (71%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 8.00 (d, 1H), 7.00-7.35 (m, 18H), 6.80 (m, 8H), 5.95 (d, 1H), 5.60 (d, 1H), 4.70 (t, 1H), 4.40 (m, 1H), 4.30 (m, 1H), 3.80 (s, 6H), 3.75 (s, 3H), 3.70 (s, 3H), 3.50 (d, 1H), 3.40 (d, 1H), 3.25 (m, 2H), 2.85 (m, 2H), 2.15 (s, 3H), 1.00 (t, 6H).

Anal. Calcd. for $C_{58}H_{60}FN_3O_{10}S$ (1010.29): C, 68.96; H, 5.99; N, 4.16. Found: C, 68.59; H, 5.92; N, 4.04.

2,5'-Anhydro-2',3'-di-*O*-*t*-butyldimethylsilyluridine (**38**).

A solution of 2,5'-anhydrouridine [18] (1.81 g, 8 mmoles) and imidazole (3.27 g, 48 mmoles) in pyridine (100 ml) was treated with *t*-butyldimethylsilyl chloride (3.62 g, 24 mmoles) and stirred at room temperature overnight. The mixture was evaporated to dryness and the resulting residue was partitioned between chloroform (250 ml) and water (100 ml). The combined organic layers were dried over sodium sulfate and evaporated to yield a yellow oil. The oil was purified by flash column chromatography (5 x 15 cm, ethyl acetate/methanol, 19:1) to yield 2.36 g (64%) of a colorless foam. This material was used without further purification; ^{1}H nmr (deuteriochloroform): δ 7.25 (d, 1H), 6.15 (d, 1H), 5.25 (s, 1H), 4.60 (m, 1H), 4.45 (m, 2H), 4.40 (d, 1H), 4.20 (d, 1H), 0.95 (s, 9H), 0.90 (s, 9H), 0.15 (m, 6H), -0.10 (s, 6H).

2,3-Dihydro-2-(2-oxohexylidene)-1-(2,3-di-*O*-*t*-butyldimethylsilyl-β-D-ribofuranosyl)-4(1*H*)-pyrimidinone (**39**).

A solution of 2-hexanone (0.63 ml, 5.12 mmoles) in tetrahydrofuran (30 ml) was cooled to -70°. Lithium diisopropylamide (2.6 ml of a 2 *M* solution in tetrahydrofuran, Aldrich) was added and the solution was stirred at this temperature for 20 minutes and then warmed to room temperature. A solution of compound **38** (0.78 g, 1.71 mmoles) in tetrahydrofuran (5 ml) was added and gave an immediate yellow color. Tlc after 45 minutes indicated complete reaction. The reaction mixture was quenched with the addition of water (10 ml), neutralized carefully with 1 *N* hydrochloric acid and then partitioned between ether (150 ml) and water (50 ml). The organic layer was separated, dried over sodium sulfate and evaporated to give a foam which was purified by flash column chromatography (3 x 15 cm; hexanes/ethyl acetate, 1:2) yielded 0.68 g (71%) of a yellowish foam; ^{1}H nmr (deuteriochloroform): δ 14.05 (s, 1H), 8.00 (d, 1H), 5.70 (d, 1H), 5.50 (d, 1H), 5.05 (s, 1H), 4.10 (m, 3H), 4.00 (dd, 1H), 3.80 (dd, 1H), 2.30 (m, 2H), 1.60 (m, 2H), 1.30 (m, 2H), 0.90 (m, 21H), 0.00-0.10 (m, 12H).

Anal. Calcd. for $C_{27}H_{50}N_2O_6Si_2$ (554.9): C, 58.44; H, 9.08; N, 5.05. Found: C, 58.28; H, 9.20; N, 4.90.

2,3-Dihydro-2-(2-oxohexylidene)-1-β-D-ribofuranosyl-4(1*H*)-pyrimidinone (**40**).

A solution of **39** (0.65 g, 1.17 mmoles) in tetrahydrofuran (30 ml) was treated with tetrabutylammonium fluoride hydrate (0.77 g, 2.93 mmoles) and stirred at room temperature overnight. The mixture was evaporated to dryness and the resulting residue was purified by flash column chromatography (3 x 15 cm, ethyl acetate/methanol, 19:1) to yield 0.22 g (57%) of a colorless foam; ^{1}H nmr (DMSO-d_6): δ 14.00 (s, 1H), 8.10 (d, 1H), 5.70 (m, 2H), 5.35 (s, 1H), 5.20 (m, 3H), 4.00 (m, 3H), 3.70 (dd, 1H), 3.60 (dd, 1H), 2.25 (m, 2H), 1.50 (m, 2H), 1.30 (m, 2H), 0.85 (t, 3H).

Anal. Calcd. for $C_{15}H_{22}N_2O_6 \cdot 0.25H_2O$ (330.9): C, 54.45; H, 6.78; N, 8.47. Found: C, 54.69; H, 6.89; N, 8.33.

REFERENCES AND NOTES

[1] Postdoctoral Associate 1991-1993.

[2a] J. S. Cohen, ed, Oligodeoxynucleotides. Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, FL (1989); [b] E. Uhlmann and A. Peyman, *Chem. Rev.*, **90**, 543 (1990); [c] J. N. M. Mol and A. R. van der Krol, eds, Antisense Nucleic Acids and Proteins. Fundamentals and Applications, Marcel Dekker, New York, NY (1991); [d] P. D. Cook, *Anticancer Drug Design*, **6**, 585 (1991); [e] S. T. Crooke, *Annu. Rev. Pharmacolol. Toxicol.*, **32**, 329 (1992); [f] P. D. Cook, Antisense Research and Applications, S. T. Crooke and B. LeBleu, eds, CRC Press, Boca Raton, FL, 1993.

[3] A. Rosenthal and R. H. Dodd, *Carbohydr. Res.*, **78**, 33 (1980).

[4] T. Kunieda and B. Witkop, *J. Am. Chem. Soc.*, **91**, 7751 (1969); [b] T. Kunieda and B. Witkop, *J. Am. Chem. Soc.*, **93**, 3478 (1971).

[5] H. Yamanaka, S. Konno, T. Sakamoto, S. Niitsuma and S. Noji, *Chem. Pharm. Bull.*, **29**, 2837 (1981).

[6] H. Vorbrüggen and K. Krolikiewicz, *Angew. Chem., Int. Ed. Engl.*, **15**, 689 (1976).

[7] H. Vorbrüggen, K. Krolikiewicz and B. Bennua, *Chem. Ber.*, **114**, 1234 (1981).

[8] B. Roth, R. Laube, M. Y. Todwell and B. Rauckmann, *J. Org. Chem.*, **45**, 3651 (1980).

[9a] S. Gabriel, *Ber.*, **37**, 3638 (1904); [b] E. Northey, *Chem. Abstr.*, **41**, 3823h (1987).

[10a] C. Chavis, F. Dumont, R. Wightman, J. C. Ziegler and J.-L. Imbach, Nucleic Acid Chemistry, Part **3**, R. S. Tipson and L. B. Townsend, eds, John Wiley and Sons, New York, NY, 1986, p 76; [b] F. Dumont, R. Wightman, J.-C. Ziegler, C. Chavis and J.-L. Imbach, *Tetrahedron Letters*, 3291 (1979); [c] C. Chavis, F. Dumont and J.-L. Imbach, *J. Carbohydrates, Nucleosides Nucleotides*, **5**, 133 (1978).

[11] A. Hampton and A. Nichol, *Biochemistry*, **5**, 2076 (1966).

[12] K. Watanabe, C. Chu, U. Reichman and J. Fox, Nucleic Acid Chemistry, R. S. Tipson and L. B. Townsend, eds, John Wiley and Sons, New York, NY, 1978, p 343.

[13] E. Pretsch, W. Simon, J. Seibl, T. Clerc and K. Biemann, Spectral Data for Structure Determination of Organic Compounds, Second Edition, W. Fresnius, J. F. K. Huber, E. Pungor, G. A. Rechnitz, W. Simon and Th. S. West, eds, Springer-Verlag, Berlin, 1989, p H50.

[14] W. Saenger, Principles of Nucleic Acid Structure, Charles R. Cantor, ed, Springer-Verlag, New York, 1984, Chapter 6.

[15] W. C. Still, M. Kahn and A. Mitra, *J. Org. Chem.*, **43**, 2923 (1978).

[16] H. Vorbrüggen and P. Strehlke, *Chem. Ber.*, **106**, 3039 (1973).

[17] K. Watanabe, U. Reichmann, C. Chu and J. Fox, Nucleic Acid Chemistry, Volume 1, R. S. Tipson and L. B. Townsend, eds, John Wiley and Sons, New York, NY, 1978, p 273.

[18a] S. Shibuya, A. Kuninaka and H. Yoshino, *Chem. Pharm. Bull.*, **22**, 719 (1974); [b] J. Kimura, Y. Fukisawa, T. Sawada and O. Mitsunobu, *Chem. Letters*, 691 (1974).