Synthesis of C-4 Substituted Pyrimidine Nucleoside Analogs. Preparation of Several 4-(2-Oxoalkylidene)-2(1*H*)-pyrimidinone Ribonucleosides

Oscar L. Acevedo*, Robert S. Andrews, Martin Dunkel and P. Dan Cook

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

Received December 31, 1993

This manuscript is dedicated to Professor Leroy B. Townsend on the occassion of his sixtieth birthday.

A series of 4-(1-alkynyl)-2(1*H*)-pyrimidinone ribonucleosides were synthesized from the Pd-catalyzed coupling of terminal alkynes to the 4-chloropyrimidin-2-one ribonucleoside (**2**). These compounds were hydrated, using three different methods, to afford the 4-(2-oxoalkylidene)-2(1*H*)-pyrimidinones. The 4-enol-pyrimidin-2-one structure of the title compounds offers functional groups with the potential for Watson-Crick hydrogen bonding.

J. Heterocyclic Chem., **31**, 989 (1994).

Introduction.

A recent report from our laboratories [1] has described efforts to develop nucleosides with tethered functionalities extending from the C-2 carbon of pyrimidines. Incorporation of these bases into antisense oligonucleotides [2] would allow the introduction of various functionalities into the minor groove of an antisense DNA:mRNA heteroduplex [3]. In a parallel effort, we have begun a program to synthesize other pyrimidines with tethered functionalities extending into the major groove of this heteroduplex. Specifically, we desired to substitute the C-4 keto group of a pyrimidine with alkyl tethers while maintaining groups which would support Watson-Crick hybridization. An energy minimized molecular model, using a BioSym modeling program, revealed that the orientation of tethers extending from C-4 of a pyrimidine would allow a shorter access to the bases on a complementary strand compared to similar tethers extending from C-5.

In this article, we present a novel synthesis of a variety of 4-(2-oxoalkylidene)-2(1*H*)-pyrimidinone ribonucleosides, using a palladium-catalyzed coupling of terminal alkynes [4] to a 4-chloro-2(1*H*)-pyrimidinone ribonucleoside [5]. The triple bonds of the 4-(1-alkynyl)-2(1*H*)-pyrimidinone ribonucleoside intermediates were hydrated, using three different conditions, to afford the title compounds in moderate yields. These pyrimidines, because of the enolized nature of their C-4 groups, contain an enol hydrogen which has potential for Watson-Crick hydrogen bonding (Figure 1).

Results and Discussion.

We envisioned a synthesis of the 4-(2-oxoalkylidene)-2(1*H*)-pyrimidinones beginning with the palladium catalyzed coupling of terminal alkynes to a 4-halopyrimidin-2-one ribonucleoside. Subsequent hydration of the alkyne products would yield the required 4-(2-oxoalkylidene)-2(1*H*)-pyrimidinones. In order to establish a synthesis of the title pyrimidines which would allow for the convergent incorporation of various 2-substituted ribofuranoses, we chose to use the 4-chloro-2-trimethylsilylethoxypyrimidine (**1**) as our starting material. We prepared **1** by a selective displacement of the 2-chloro group of 2,4-dichloropyrimidine using trimethylsilylethanol/*n*-BuLi in tetrahydrofuran at -68°. The pattern of substitution on 2,4-dichloropyrimidine with this alkoxide is contrary to reports in the literature [6] that describe 4-methoxy-2-chloropyrimidine as the sole product of reaction between 2,4-dichloropyrimidine and sodium methoxide in methanol. The reversal of reactivity between the C-2 and C-4 positions here may be due to an electrostatic effect [7] that promotes complexation of lithium to the more basic N-1 nitrogen. In an aprotic solvent like tetrahydrofuran, this complexation may facilitate reaction of the trimethylsilylethoxide at the 2-position to yield **1**. Pyrimidine **1** was reacted with 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose under standard Vorbrüggen

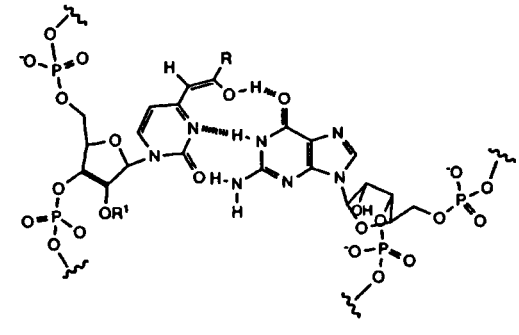
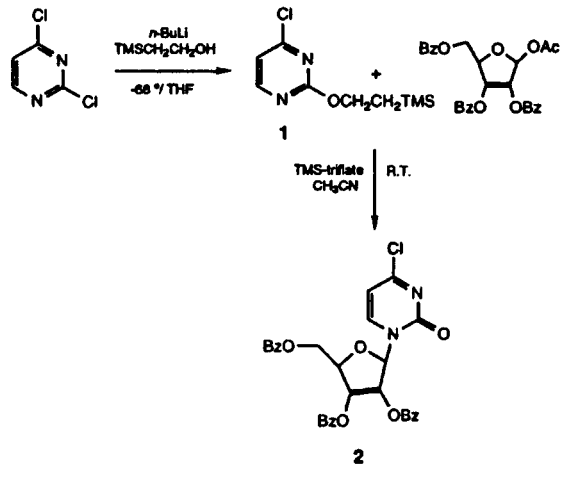


Figure 1. Proposed hydrogen bonding scheme for 4-(2-Oxoalkylidene)-2(1*H*)-pyrimidinone nucleoside analogs with guanosine.

conditions [8] to afford excellent yields of the 4-chloropyrimidine-2-one ribonucleoside **2**.

Scheme 1

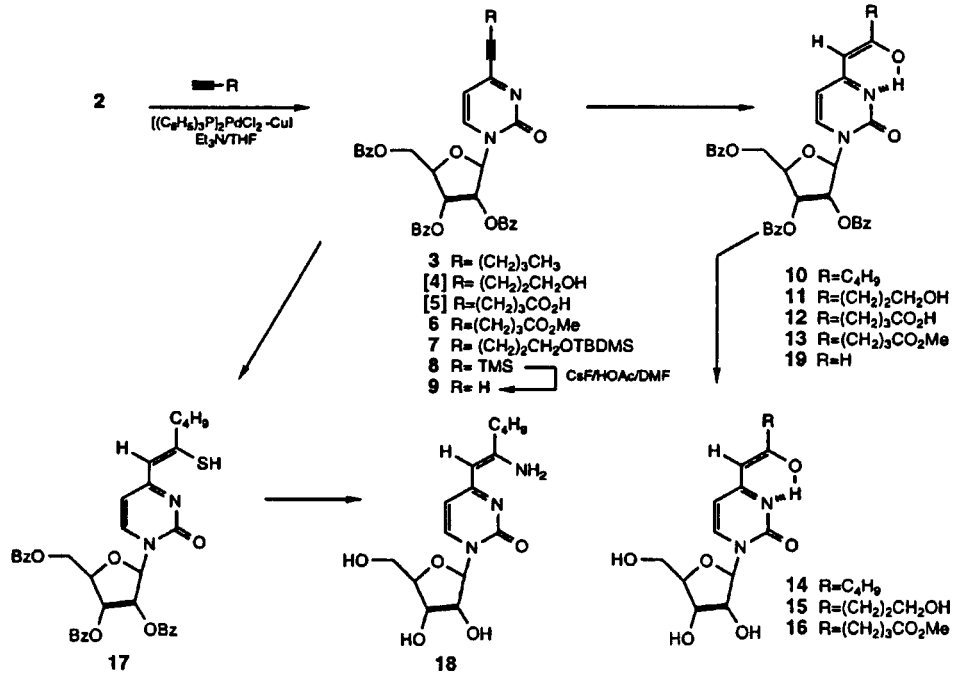


Further, it was found that **2** was an excellent ligand in palladium-catalyzed coupling to various terminal alkynes [4]. For example, the reaction of **2** with 1-hexyne in the presence of $[(C_6H_5)_3P]_3Pd(II)Cl_2$-Cu(I) afforded 4-(1-hexynyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**3**) at room temperature. This material was isolated following a simple aqueous workup. Likewise, a Pd-catalyzed reaction of **2** with trimethylsilylacetylene yielded the 4-(trimethylsilylethynyl)-2(1*H*)-pyrimidinone (**8**) in good yield. Coupling of **2** with 5-hexynoic acid or 4-pentyn-1-ol yielded products **4** and **5** which hydrated during aqueous workup of their respective reaction mixtures [9]. Thus, the desired 4-(2-oxopentenyl-5-ol) and 4-(2-oxo-6-carboxypentenyl)-4(1*H*)-pyrimidinones **11** and **12**, respectively were obtained directly from **2**. Evidence for the presence of compounds **4** and **5** was seen on thin layer chromatography plates previous to workup of their individual reaction mixtures with dilute acid. We were not able to effect a similar *in situ* hydration of compounds **3** and **8** under identical workup conditions.

The hydration of **3** was effected using mercuric sulfate-acetic acid [10] in an aqueous acetone medium, affording the required 2-(2-oxohexenyl)-4(1*H*)-pyrimidinone (**10**) in only 59% yield. This yield was undoubtedly reduced by the inevitable hydrolysis of the nucleoside bond during a reaction which required 18 hours for completion. However, the method of Chapdelaine employing sodium hydrogen sulfide-aqueous HCl [11] and much shorter reaction times gave **10** in excellent yield. The ^1H nmr spectrum of **10** was nearly identical with the spectra of compounds **11-13**, thus suggesting a common orientation of hydration for the compounds in this series.

In contrast, the hydration of compound **8** using mercuric sulfate in acetic acid yielded only products of nucleoside hydrolysis. The difficulty in hydrating the alkyne group of **8** was attributed to the insolubility of this compound in the aqueous acid-acetone mixtures required for this transformation. Consequently, the trimethylsilyl group was first removed using cesium fluoride in acetic

Scheme 2



acid-DMF to yield the intermediate alkyne **9**. Using the hydration conditions of Chapdelaine [10], this compound then underwent a smooth transformation to a material which had incorporated a molar equivalent of water. We presume that under these conditions of hydrolysis a nucleoside β-enethiol is formed [12] and that this intermediate hydrolyzes in the presence of strong acid to yield the 4-(2-oxoethenyl) group of **19** [13]. This transformation could also be effected by the prolonged treatment of **8** with acetic acid in DMF-acetone. Unfortunataly, repeated efforts to deprotect **19** yielded only materials which decomposed rapidly during isolation.

The alkyne **3** is an excellent electrophile: for instance, **3** was reacted with methanolic ammonia to afford the enamine **18** in good yield. In the ^{1}H nmr of **18**, the vinyl proton of the tether appears as sharp singlet at 4.75 ppm and the enamine proton signals are split at 10.2 and 7.7 ppm, consistent with a hydrogen bond of the downfield enamine proton to N-3 of the ring. Likewise, crude reaction mixtures which gave **3** were treated *in situ* with hydrogen sulfide gas to yield the enethiol **17**. The ^{1}H nmr spectrum of this bright reddish compound exhibits a thiol signal at 15.5 ppm and a uv maximum at 358 nm. The regioisomer indicated is the result of Michael addition of sulfide to compound **3** [14]. The enethiol group of **17** was easily displaced by ammonia to yield the enamine **18** as the sole product.

Coupling of **2** with propargyl alcohol or its methyl ether could not be effected under these conditions nor in the presence of a CuI and *tetrakis*-triphenylphosphine palladium(0) catalyst mixture. Likewise, no product could be isolated from the coupling reaction of **2** with ethyl propiolate or propargylaldehyde dimethylacetal. A gentle heating of these reaction mixtures caused decomposition of the chloropyrimidine nucleoside starting material.

The palladium-catalyzed coupling of terminal alkynes to the 4-chloropyrimidinone **2** has produced several interesting results. First, the coupling gives good to excellent yields of the expected products, all under mild conditions. Second, the *in-situ* hydration of the triple bond of some of the products occured during workup with dilute aqueous acid. This hydration occurred only for those compounds that contained an ionizable group on the tether, *e.g.* hydroxy or carboxylate. Thus, the triple bond of the 4-trimethylsilyl-acetylide **8** and the 4-hexynyl **3** remained intact, whereas identical workup of **4** and **5** yielded the enol-alcohol **11** and enol-acid **12**. To further demonstrate this effect, the coupling of **2** and a methyl 5-hexynoate was carried out to yield a product with an intact triple bond, compound **6**. Likewise, when the alcohol of 4-pentyn-1-ol was protected with a *t*-butyldimethylsilyl group, coupling of the protected alkyne also yielded a product with an intact triple bond, **7**. Though the mechanism of the hydration was not investigated further, this finding implies that ionizable groups assist in the hydration of the triple bonds of **4** and **5**. However, a simpler explanation may be that these groups impart added miscibility in acid of the aqueous acid-tetrahydrofuran-chloroform mixtures present during workup.

Third, hydration of the 4-alkynyl groups of compounds **3-6** and **9** has given 2-oxoalkylidene products **10-13** and **19**. This orientation is not normally observed from acid- or mercuric ion-promoted hydrations, which usually proceed in Markownikoff fashion [15]. The *E*-geometry assigned to the 4-(2-oxoalkylidene)-2(1*H*)-pyrimidinones is based on the 2D-noesy experiments performed on **10** and **11** which showed strong nOe cross-peaks for the vinylic and the H-5 resonances. In each case, the enol hydrogen appears far downfield (*ca* 12.5 ppm) indicating strong hydrogen bonding to N-3 [16]. The 2-oxo configuration was determined based on nmr experiments which revealed strong ^{1}H-^{13}C nOe enhancement of the enolic carbon from irradiation of the allyl hydrogens of the tether. Likewise, irradiation of the vinylic proton yielded enhancement of the enolic and C-4 carbons, but not of the allyl carbon. Both are virtually locked in the *E* configuration, though keto-enol tautomerism could establish a mixed population of *E* and *Z* isomers.

Previous work in this area is limited to Vorbrüggen [13] who developed a simple sulfur-extrusion method for the placement of a phenacyl group at the 4-position of uridine. The 4-(2-oxoalkylidene)-2(1*H*)-pyrimidinones reported here represent a new class of pyrimidines with versatile functionalized tethers. The hydrogen bonding scheme we envision would involve the 4-enol, N-3 and 2-keto groups as hydrogen bond donor, acceptor and acceptor respectively. In this scheme, as many as three Watson-Crick hydrogen bonds to guanosine could be formed (see Figure 1). Further, our molecular models indicate that the bulk of an alkylidene tether at this position causes little structural perturbation in an energy-minimized double helix which paired a 4-(2-oxoalkylidene)-2(1*H*)-pyrimidinone against guanosine on an RNA complementary strand. Thus, it is our next task to incorporate these nucleosides into oligonucleotides using phosphoramidite chemistries and to determine their hybridization properties. A more detailed account of the characterization of this family of compounds by nmr and of their incorporation into oligonucleotides will appear 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. Rotary evaporations were carried

out *in vacuo* at 35-45° using a vacuum pump and vacuum controller combination. 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 [17]. Solvent systems: A, chloroform-acetone, 19:1; B, ethyl acetate-hexane, 7:3; C, ethyl acetate; D, ethyl acetate-methanol, 9:1; E, ethyl acetate-methanol, 4:1. Drying of materials for analysis was achieved in a drying oven *in vacuo* at 40-60°. Elemental analyses were performed by Quantitative Technologies, Bound Brook, N. J. All Pd-coupling reactions were performed in freshly distilled tetrahydrofuran under an argon atmosphere. Other dry solvents and reagents were purchased from Aldrich unless otherwise noted.

4-Chloro-2-trimethylsilylethoxypyrimidine (**1**).

A solution of trimethylsilylethanol (10.0 g, 85 mmoles) in anhydrous tetrahydrofuran was cooled to -68° in a 2-propanol-dry ice bath and then treated with *n*-butyllithium (33.8 ml of a 2.5 *M* solution) dropwise over 30 minutes. This mixture was warmed to -25° and then added *via* cannula to a solution of 2,4-dichloropyrimidine (12.6 g, 85 mmoles) at -68° while maintaining anhydrous conditions. After addition, the mixture was allowed to warm to room temperature over 30 minutes and stirred at this temperature for 1 hour. The clear yellow solution was diluted with diethyl ether (200 ml) and then washed with cold water and cold, saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate and evaporated to yield a thick amber oil. This oil was kept under vacuum overnight and then flash chromatographed over a silica gel column (7.5 x 8.5 cm) using chloroform as eluent. The solvent was thoroughly evaporated and the resulting yellowish oil was precipitated at -68° from a minimum volume of hexane. Waxy solid. Yield is 20.3 g (91%); ^{1}H nmr (DMSO-d_6): 8.55 (d, 1, H-6, $J_{6,5}$ = 4.5 Hz), 7.27 (d, 1, H-5), 4.40 (t, 2, CH_2), 1.08 (t, 2, CH_2), 0.10 (s, 9, trimethylsilyl).

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

A solution of **1** (8.17 g, 35.4 mmoles) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-D-ribose (35.4 mmoles) in anhydrous acetonitrile (200 ml) was treated with trimethylsilyl triflate (6.10 ml, 31.6 mmoles) at room temperature and under a dry argon atmosphere. A rapid evolution of gas was followed by a slight darkening of the solution after 1.5 hour. The mixture was evaporated to an amber semi-solid which was immediately dissolved in 300 ml of methylene chloride. This solution was washed sequentially with cold sodium bicarbonate, water and brine. The organic solvent was removed by rotary evaporation and the cream colored residue which resulted was triturated with cold acetonitrile and filtered. The filter cake was washed with additional cold acetonitrile and dried in a vacuum oven at room temperature. Yield is 14.6 g (72%); ^{1}H nmr (DMSO-d_6): 8.40 (d, 1, H-6, $J_{6,5}$ = 7 Hz), 8.0-7.4 (m, 15, aromatic), 6.67 (d, 1, H-5), 6.21 (bs, 1, H-1'), 6.95 (m, 2, H-2',3'), 4.8 (m, 3, H-4',5',5").

Anal. Calcd. for $C_{30}H_{23}N_2O_8Cl$ (574.97): C, 62.67; H, 4.03; N, 4.87. Found: C, 62.41; H, 3.92; N, 4.80.

General Procedure: Palladium-catalyzed Coupling. 4-(1-Hexynyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**3**).

A suspension of *bis*-triphenylphosphinepalladium(II) chloride (0.35 g) and copper(I) iodide (0.095 g) in anhydrous tetrahydrofuran (150 ml) was purged with argon gas for 10 minutes at room temperature. The suspension was treated with triethylamine (5 ml) and 1-hexyne (2.5 g, 30 mmoles) under an argon atmosphere, which produced a dark amber color after 5 minutes. To this dark solution was added **2** (5.74 g, 10 mmoles) as a finely ground powder. Tlc (chloroform-acetone, 98:2, double development) of the reaction mixture after 90 minutes indicated complete conversion to a product which migrated just faster than starting material. The reaction mixture was diluted with 300 ml of chloroform and then sequentially washed with cold 1 *N* hydrochloric acid, water and brine. The organic layer was dried over magnesium sulfate and evaporated to yield a tan solid. This solid was triturated with diethyl ether and filtered to yield a white amorphous powder. Yield is 5.46 g (88%); ^{1}H nmr (DMSO-d_6): 8.33 (d, 1, H-6, $J_{6,5}$ = 6.5 Hz), 8.1-7.4 (m, 15, aromatic), 6.54 (d, 1, H-5), 6.24 (bs, 1, H-1'), 6.00 (m, 2, H-2',3'), 4.9-4.7 (m, 3, H-4',5',5"), 3.1, 1.5, 1.2 and 0.9 (4 m, 9, butyl).

Anal. Calcd. for $C_{36}H_{32}N_2O_8$ (620.66): C, 69.67; H, 5.20; N, 4.51. Found: C, 69.32: H, 5.13; N, 4.44.

Methyl 5-Hexynoate.

A solution of 5-hexynoic acid (5 g, 44.6 mmoles, purchased from Lancaster Synthesis) in anhydrous methanol (40 ml) was treated with trimethylsilyl chloride (12.5 ml, 99 mmoles) dropwise over ten minutes. The mixture was stirred at 40° for 2 hours and then the excess methanol was removed by rotary evaporation (water bath 30-33°, 100 mbar). The oil which remained was distilled by Kugelrohr apparatus (65°, 5 mbar) to afford a colorless oil. Yield is 3.9 g (70%); ^{1}H nmr (deuteriochloroform): 3.65 (s, 3), 2.5-1.7 (m, 7).

4-(Methyl-1-pentynoate)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**6**).

A coupling of **2** (5.75 g, 10.0 mmoles) and methyl 5-hexynoate (2.5 g, 20 mmoles) was performed according to the General Procedure described above. After 2 hours, the reaction mxture was diluted with chloroform (200 ml) and then sequentially washed with 1 *N* HCl, water and brine. The organic extracts were dried over sodium sulfate, filtered and coevaporated onto silica gel (10 g). This material was flash chromatographed on a silica gel column (5.5 x 15 cm) using solvent system B. The appropriate fractions were collected and evaporated to afford an amorphous yellow solid. Yield is 3.3 g (50%); ^{1}H nmr (deuteriochloroform): 8.2-7.3 (m, 16, aromatic and H-6), 6.47 (d, 1, H-1', $J_{1',2'}$ = 4.7 Hz), 6.22 (d, 1, H-5, $J_{5,6}$ = 7.5 Hz), 5.85 (m, 2, H-2',3'), 4.70 (m, 3, H-4',5',5"), 3.70 (s, 3, methyl), 2.55 (m, 4, 2 CH_2), 0.95 (m, 2, CH_2).

Anal. Calcd. for $C_{36}H_{32}N_2O_{10}$ (652.66): C, 66.86; H, 4.85; N, 4.21. Found: C, 66.66; H, 4.82; N, 4.15.

4-Pentyn-1-*O*-*t*-butyldimethylsilyl Ether.

A solution of 4-pentyn-1-ol (3.1 g, 36 mmoles) and imidazole (5.0 g, 72 mmoles) in dry dichloromethane (30 ml) was treated with *t*-butyldimethylsilyl chloride (5.5 g, 36 mmoles) at room temperature. The mixture was stirred under anhydrous conditions for 30 minutes and then treated with cold, saturated sodium bicarbonate solution. The organic layer was further washed with

cold 1 *N* HCl, dried over sodium sulfate and evaporated to afford a light yellow oil. This oil was distilled on a Kugelrohr apparatus (5 mbar, 30°) to yield a colorless oil. Yield is 4.3 g (60%); ^{1}H-nmr (deuteriochloroform): 3.65 (t, 2, CH_2), 2.25 (m, 2, CH_2), 1.90 (t, 1, alkyne), 1.70 (m, 2, CH_2), 1.85 (s, 9, *t*-butylsilyl), 0.3 (s, 6, dimethylsilyl).

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

A coupling of **2** (4.53 g, 7.90 mmoles) and 4-pentyn-1-*t*-butyldimethylsilyl ether (3.13 g, 15.8 mmoles) was performed according to the General Procedure described above. At the end of 90 minutes, the reaction was diluted with chloroform (200 ml) and the mixture was washed with cold 1 *N* HCl, water and brine. The organic layer was dried over sodium sulfate and evaporated to yield a dark oil which was flash chromatographed on a silica gel column (6 x 8 cm) using chloroform-acetone (97:3) to yield a yellowish foam. Yield is 3.30 g (57%); ^{1}H-nmr (deuteriochloroform): 8.2-7.3 (m, 15, aromatic and H-6), 6.45 (d, 1, H-1', $J_{1',2'}$ = 4.4 Hz), 6.23 (d, 1, H-5, $J_{5,6}$ = 5.0 Hz), 5.85 (m, 2, H-2',3'), 4.80 (m, 3, H-4',5',5"), 3.70, 2.55 and 1.70 (3 m, 6, -$CH_2CH_2CH_2$-), 0.90 (s, 9, *t*-butylsilyl), 0.06 (s, 6, dimethylsilyl).

Anal. Calcd. for $C_{41}H_{44}N_2O_9Si$ (736.90): C, 66.82; H, 6.02; N, 3.80. Found: C, 66.48; H, 5.99; N, 3.69.

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

A coupling of **2** (5.75 g, 10.0 mmoles) and trimethylsilylacetylene (4.91 g, 50 mmoles) was performed according to the General Procedure described above. The reaction was complete after 45 minutes, by which time a flocculent precipitate had appeared. The mixture was diluted with 100 ml of diethylether and then filtered. The filter cake was dissolved in 200 ml of chloroform and then sequentially washed with cold 1 *N* hydrochloric acid, water and brine. The chloroform was evaporated to yield a white amorphous powder. Yield is 5.12 g (80%); ^{1}H nmr (deuteriochloroform): 8.10 (d, 1, H-6, $J_{6,5}$ = 7.5 Hz), 8.0-7.3 (m, 15, aromatic), 6.45 (d, 1, H-5), 6.28 (bs, 1, H-1'), 5.90 (m, 1, H-2'), 5.80 (m, 1, H-3'), 4.9-4.7 (m, 3, H-4',5',5"), 0.25 (s, 9, trimethylsilyl).

Anal. Calcd. for $C_{35}H_{32}N_2O_8Si$ (636.73): C, 66.02; H, 5.07; N, 4.40. Found: C, 65.68; H, 4.93; N, 4.39.

4-Ethynyl-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**9**).

A solution of compound **8** (4.07 g, 6.4 mmoles) in a mixture of tetrahydrofuran-dimethylformamide (100 ml, 1:1) was treated with glacial acetic acid (3 ml) followed by anhydrous cesium fluoride (1.3 g, 8.6 mmoles). The mixture was stirred at room temperature for 3 hours and then quenched with the addition of cold, saturated sodium bicarbonate solution (30 ml). The mixture was extracted with dichloromethane (3 x 50 ml) and the combined organic extracts were dried over sodium sulfate and evaporated to dryness to afford a tan semi-solid. This material was triturated with cold acetonitrile and filtered to afford a tan amorphous powder. Yield is 3.14 g (87%); ^{1}H-nmr (deuteriochloroform): 8.1-7.3 (m, 16, aromatic and H-6), 6.43 (d, 1, H-1', $J_{1',2'}$ = 3 Hz), 6.30 (d, 1, H-5, $J_{5,6}$ = 7.4 Hz), 5.88 (m, 1, H-2'), 5.80 (m, 1, H-3'), 4.9-4.7 (m, 3, H-4',5',5"), 3.43 (s, 1, ethynyl).

Anal. Calcd. for $C_{32}H_{24}N_2O_8$ (564.55): C, 68.08; H, 4.29; N, 4.96. Found: C, 67.68; H, 4.28; N, 4.81.

4-(2-Oxohexenyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**10**).

The compound **3** (0.76 g, 1.23 mmoles) was dissolved in acetone (30 ml) by warming and treated with a solution of mercuric acetate (12.5 ml of 0.1 *M* mercuric sulfate in 8 *N* acetic acid). This solution was stirred at room temperature for 18 hours. The dark mixture was diluted with chloroform (150 ml) and then washed with cold, saturated sodium bicarbonate, water and brine. The organic layer was dried over sodium sulfate and then evaporated to yield an orange foam. This foam was flash chromatographed on a silica gel column (3.5 x 8 cm) using solvent system A. The appropriate fractions were evaporated to yield a light yellow foam. This foam was triturated with cold methanol to afford a light yellow amorphous powder. Yield is 0.46 g (59%); ^{1}H nmr (DMSO-d_6): 12.5 (s, 1, enol, exchanged with deuterium oxide), 8.0-7.4 (m, 16, aromatic and H-6), 6.15 (bs, 1, H-1'), 5.90 (m, 3, H-5,2',3'), 5.39 (s, 1, vinylic), 4.68 (m, 3, H-4',5',5"), 2.30, 1.47, 1.25 and 0.87 (4 m, 9, butyl).

Anal. Calcd. for $C_{36}H_{34}N_2O_9$ (638.67): C, 67.70; H, 5.37, N, 4.39. Found: C, 67.51; H, 5.28; N, 4.28.

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

A coupling of **2** (10.8, 18.9 mmoles) and 4-pentyn-1-ol (3.18 g, 37.8 mmoles) under the catalysis of *bis*-triphenylphosphinepalladium(II) chloride (0.66 g) and copper(I) iodide (0.18 g) was carried out according to the General Procedure described above. Tlc (ethyl acetate) indicated that the reaction was complete after 75 minutes and the mixture was then diluted with 350 ml of chloroform. This solution was then washed with cold 1 *N* HCl, water and brine, then dried over magnesium sulfate. The chloroform was evaporated to yield an amber syrup which was triturated with cold diethyl ether to yield an off-white solid. This material was used without further purification. Yield is 11.8 g (98%). A portion of this material (1 g) was flash-chromatogfaphed on a silica gel column (3.5 x 5 cm) column using solvent C to obtain an analytical sample, 0.70 g; ^{1}H nmr (DMSO-d_6): 12.5 (s, 1, enol, exchanged with deuterium oxide), 8.1-7.5 (m, 16, aromatic and H-6), 6.18 (bs, 1, H-1'), 5.95 (m, 3, H-5,2',3'), 5.42 (s, 1, vinylic), 4.76 (m, 3, H-4',5',5"), 4.1 (bs, 1, hydroxy, exchanged with deuterium oxide), 3.4, 2.4 and 1.68 (3 m, 6, -$CH_2CH_2CH_2$-).

Anal. Calcd. for $C_{35}H_{32}N_2O_{10}$ (640.65): C, 65.62; H, 5.03; N, 4.37. Found: C, 65.32; H, 4.99; N, 4.21.

4-(2-Oxo-6-carboxypentylidene)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)pyrimidinone (**12**).

A coupling of **2** (10.8 g, 18.9 mmoles) and 5-hexynoic acid (4.3 g, 38.3 mmoles) under the catalysis of *bis*-triphenylphosphinepalladium(II) chloride (0.66 g) and copper(I) iodide (0.18 g) was performed according to the General Procedure described above. After 2 hours, the reaction mixture was diluted with chloroform (350 ml) and washed sequentially with cold 1 *N* hydochloric acid, 0.01 *M* aqueous ethylenediamine tetraacetic acid, water and brine, then dried over magnesium sulfate. The chloroform was evaporated to yield a reddish solid which was repeatedly triturated with cold diethylether and filtered. Yield is 12.0 g (97%). A 1 g sample of this material was flash chromatographed on silica gel column (5.5 x 4 cm) using solvent system C to give a sample for nmr; 0.70 g; ^{1}H-nmr (DMSO-d_6): 12.45 and 12.03 (2 s, 2, hydroxy, carboxylic, exchanged with

deuterium oxide), 8.0-7.4 (m, 15, aromatic and H-6), 6.15 (s, 1, H-1'), 5.90 (m, 3, H-5,2',3'), 5.38 (s, 1, vinylic), 4.44 (m, 3, H-4',5',5"), 2.35, 2.22 and 1.68 (3 m, 6, $-CH_2CH_2CH_2-$). This material was used as such without further purification.

4-(Methyl 2-oxohexenoate)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**13**).

A solution of **6** (0.75 g, 1.2 mmoles) in acetone-dimethylformamide (18 ml, 5:1) was treated with a solution of sodium hydrogen sulfide (2.3 ml of a 0.1 *N* aqueous solution) followed by a dilute hydrochloric acid solution (1.2 ml of a 3 *N* aqueous solution) at room temperature. The mixture was stirred for 2 hours and then quenched with the addition of cold saturated sodium bicarbonate (20 ml). The mixture was then further diluted with dichloromethane (50 ml) and the organic layer was washed with cold aqeuous sodium bicarbonate, water and brine, then dried over sodium sulfate and evaporated to afford an orange syrup. This material was flash chromatographed on silica gel using chloroform-acetone (97:3) to afford an orange glass. Yield is 0.59 g (77%); ^{1}H-nmr (deuteriochloroform): 12.4 (s, 1, enol, exchanged with deuterium oxide); 8.2-7.2 (m, 15, aromatic), 7.00 (d, 1, H-6, $J_{6,5}$ = 5.9 Hz), 6.60 (d, 1, H-1', $J_{1',2'}$ = 4.9 Hz), 5.87 (m, 1, H-3'), 5.70 (m, 1, H-2'), 5.48 (d, 1, H-5), 5.15 (s, 1, vinylic), 4.70 (m, 3, H-4',5',5"), 3.68 (s, 3, methyl ester), 2.35 and 1.95 (2 m, 6, $-CH_2CH_2CH_2-$). This material was used as such without further purification.

4-(2-Oxohexenyl)-1-β-D-ribofuranosyl-4(1*H*)-pyrimidinone (**14**).

A suspension of **10** (0.51 g, 0.8 mmole) and powdered potassium carbonate (50 mg) over absolute ethanol (20 ml) was stirred under anhydrous conditions and at room temperature for 18 hours. The mixture was filtered and the filtrate coevaporated onto silica gel (1.5 g). This material was flash chromatographed on a silica gel column (3.5 x 4 cm) using solvent system D. The appropriate fractions were collected and evaporated to yield a yellowish foam. Yield is 0.15 g (57%); ^{1}H-nmr (DMSO-d_6): 12.5 (bs, 1, enol, exchanged with deuterium oxide), 7.62 (d, 1, H-6, $J_{6,5}$ = 7.4 Hz), 5.95 (d, 1, H-5), 5.80 (d, 1, H-1', $J_{1',2'}$ = 5.4 Hz), 5.40 (m, 2, hydroxyl and vinylic, simplifies to singlet with deuterium oxide exchange), 5.10 (m, 2, hydroxyls, exchanged with deuterium oxide), 4.00 (m, 3, H-4',5',5"), 2.32, 1.50, 1.28 and 0.90 (4 m, 9, butyl).

Anal. Calcd. for $C_{15}H_{22}N_2O_6 \cdot 0.25H_2O$ (330.85): C, 54.45; H, 6.85; N, 8.47. Found: C, 54.65; H, 6.75; N, 8.34.

4-(2-Oxopentenyl-5-ol)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**15**).

A suspension of **11** (0.65 g, 1.04 mmoles) and powdered potassium carbonate (60 mg) over absolute ethanol (15 ml) was stirred under anhydrous conditions and at room temperature for 18 hours. The mixture was evaporated to afford a yellow residue which was partitioned between water (50 ml) and ether (25 ml). The water layer was washed with additional ether (25 ml) and then evaporated to afford a dark orange residue. This residue was coevaporated onto silica gel (1.5 g) and then flash chromatographed on a silica gel column (3.5 x 5 cm) using solvent system C (50 ml) and then E (200 ml). The appropriate fractions were collected and evaporated to afford a yellowish amorphous solid. Yield is 0.24 g (73%); ^{1}H-nmr (DMSO-d_6): 12.5 (s, 1, enol, exchanged with deuterium oxide), 7.58 (d, 1, H-6, $J_{6,5}$ = 3.4 Hz), 5.88 (d, 1, H-1', $J_{1',2'}$ = 3.5 Hz), 5.75 (d, 1, H-5), 5.40 (m, 1, H-2'), 5.35 (s, 1, vinylic), 5.05 (m, 2, H-3' and hydroxyl, simplifies with deuterium oxide exchange), 4.43, 4.00 and 3.95 (3 m, 3, hydroxyls, exchanged with deuterium oxide), 3.80 (m, 1, H-4'); 3.57 (m, 2, H-5',5"), 3.35, 2.35 and 1.63 (3 m, 6, $-CH_2CH_2CH_2-$).

Anal. Calcd. for $C_{14}H_{20}N_2O_7$ (328.32): C, 51.22; H, 6.14; N, 8.53. Found: C, 51.08; H, 6.06; N, 8.43.

4-(Methyl 2-oxohexenoate)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**16**).

A solution of **13** (0.38 g, 0.56 mmole) in methanolic ammonia (20 ml, saturated at -20°) was stirred at room temperature for 18 hours. The resulting yellow solution was evaporated to yield an oil which was triturated with ether (3 x 15 ml) then chromatographed on two preparative chromatography plates (25 x 25 cm) using solvent system E. The appropriate band was scraped off and the material eluted with acetonitrile-methanol to yield a white amorphous solid. Yield is 0.14 g (65%); ^{1}H-nmr (DMSO-d_6): 7.64 (d, 1, H-6, $J_{6,5}$ = 7.6 Hz), 5.94 (d, 1, H-5), 5.78 (d, 1, H-1', $J_{1',2'}$ = 4.2 Hz), 5.36 (s, 1, vinylic), 5.4 and 5.1 (2 m, 3, hydroxyls, exchanged with deuterium oxide); 3.90 (m, 3, H-4',5',5"), 3.60 (s, 3, methyl ester), 2.3 and 1.7 (2 m, 6, $-CH_2CH_2CH_2-$).

Anal. Calcd. for $C_{16}H_{22}O_8 \cdot 0.5H_2O$ (379.37): C, 50.65; H, 6.11; N, 7.38. Found: C, 50.44; H, 5.83; N, 7.15.

4-(2-Thiohexenyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**17**).

A palladium-catalyzed coupling of **2** (5.75 g, 10.0 mmoles) and 1-hexyne was carried out according to the General Procedure described above. The reaction mixture was diluted with 150 ml of chloroform and purged with dry hydrogen sulfide gas for 5 minutes and then with argon gas for 10 minutes at room temperature. The mixture was dried over sodium sulfate, filtered through Celite and evaporated to afford a violet residue. The residue was cooevaporated onto silica gel (12 g) and then flash chromatographed on a silica gel column (5.5 x 15 cm) column using solvent system A. The appropriate fractions were collected and evaporated to afford an orange foam. Yield is 5.12 g (78%); ^{1}H nmr (DMSO-d_6): 15.3 (s, 1, thiol, exchanged with deuterium oxide), 8.1-7.4 (m, 16, aromatic and H-6), 6.43 (s, 1, vinylic), 6.22 (m, 2, H-1', H-5), 5.97 (m, 2, H-2',3'), 4.75 (m, 3, H-4',5',5"), 2.70, 1.65, 1.30 and 0.88 (4 m, 9, butyl).

Anal. Calcd. for $C_{36}H_{34}N_2O_8S$ (654.73): C, 66.04; H, 5.23; N, 4.27; S, 4.89. Found: C, 66.10; H, 5.18; N, 4.13; S, 4.43.

4-(2-Aminohexenyl)-1-β-D-ribofuranosyl-2(1*H*)-pyrimidinone (**18**).

Method A.

A finely ground sample of compound **3** (1.2 g, 1.93 mmoles) was suspended in methanolic ammonia (45 ml, saturated at -20°) and sealed in a stainless steel vessel. The mixture was stirred at room temperature for 18 hours; at the end of this time, tlc indicated the presence of one major product, R_f = 0.6. The clear solution was evaporated to afford a light yellow syrup which was immediately evaporated onto silica gel (1 g) using methanol and then flash chromatographed on a silica gel column (3.5 x 4 cm) using solvent system C (100 ml) and then E (200 ml). The appropriate fractions were collected and evaporated to afford a yellow amorphous solid. Yield is 0.47 g (78%); ^{1}H nmr (DMSO-d_6): 10.2 and 7.7 (2 bs, 2, NH_2, exchanged with deuterium oxide), 7.83 (d, 1, H-6, $J_{6,5}$ = 8.4 Hz), 5.90 (d, 1, H-5), 5.78 (s, 1, H-1'), 5.32, 5.05 and 5.00 (3 m, 3, hydroxyls, exchanged

with deuterium oxide), 4.75 (s, 1, vinylic), 3.95 (m, 2, H-2',3'), 3.83 (m, 1, H-4'), 3.60 (m, 2, H-5',5"), 2.18, 1.54, 1.30 and 0.88 (4 m, 9, butyl).

Anal. Calcd. for $C_{15}H_{23}N_3O_5 \cdot 0.5H_2O$ (334.37): C, 53.88; H, 7.23; N, 12.56. Found: C, 53.96; H, 7.34; N, 12.23.

Method B.

A solution of compound **17** (1.18 g, 1.80 mmoles) in methanolic ammonia (30 ml, saturated at -20°) was sealed in a stainless steel vessel and stirred at room temperature for 18 hours. The solution was evaporated and the material which resulted was triturated with cold diethyl ether (3 x 10 ml) and then crystallized from hot methanol to afford yellowish crystals. Yield is 0.47 g (78%), mp 168-170°. This material was identical in a co-spot analysis by tlc and in its nmr and elemental analysis to the material obtained from Method A.

4-(2-Oxoethenyl)-1-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)-2(1*H*)-pyrimidinone (**19**).

A solution of compound **9** (1.48 g, 2.62 mmoles) in tetrahydrofuran-dimethylformamide (40 ml, 3:1) was treated with an aqueous solution of sodium hydrogen sulfide (5.3 ml of a 0.1 *M* solution) followed by dilute hydrochloric acid (2.6 ml of a 3 *N* solution). The reaction is complete after 15 minutes at room temperature and is quenched with the addition of cold, saturated sodium bicarbonate solution (30 ml). The mixture was extracted with chloroform (3 x 75 ml) and the combined organic extracts were washed with water and brine and then dried over sodium sulfate, filtered and coevaporated onto silica gel (3 g). This material was flash chromatographed on a silica gel column (3.5 x 17 cm) using chloroform-acetone (98:2). The appropriate fractions were collected and evaporated to afford a salmon colored solid. Yield is 0.61 g (40%); ^{1}H-nmr (deuteriochloroform): 12.3 (bs, 1, enol, exchanged with deuterium oxide), 9.23 (d, 1, H-6, $J_{6,5}$ = 2.3 Hz), 8.2-7.3 (m, 15, aromatic), 7.10 (d, 1, vinylic, J_{vic} = 8.3 Hz), 6.40 (d, l, H-1', $J_{1',2'}$ = 5.4 Hz), 5.90 (m, 1, H-2'), 5.73 (m, 1, H-3'), 5.55 (d, 1, vinylic, sharpens with deuterium oxide exchange), 5.20 (d, 1, H-5), 4.75 (m, 3, H-4',5',5").

Anal. Calcd. for $C_{32}H_{26}N_2O_9$ (582.57): C, 65.98; H, 4.50; N, 4.81. Found: C, 65.83; H, 4.50; N, 4.79.

REFERENCES AND NOTES

[1] M. Dunkel, P. Dan Cook and O. L. Acevedo, *J. Heterocyclic Chem.*, 30,1421 (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] W. Saenger, Principles of Nucleic Acid Structure, Charles R. Cantor, ed, Springer-Verlag, New York, 1984, Chapter 11.

[4a] K. Sonogashira, Y. Tohda and N. Hagihara, *Tetrahedron Letters*, **50**, 4467 (1975); [b] R. F. Heck, Palladium Reagents in Organic Synthesis, A. R. Katritzky, O. Meth-Cohn and C. W. Rees, eds, Academic Press, San Diego, 1985, p 299.

[5] J. Zemlicka and F. Sorm, *Collect. Czech. Chem. Commun.*, **30**, 2052 (1965).

[6] D. J. Brown, The Pyrimidines. The Chemistry of Heterocyclic Compounds, A. Weissberger, ed, John Wiley and Sons, New York, 1962, p 201.

[7] R. G. Shepherd and J. L. Fredrick, Reactivity of Azine, Benzoazine, and Azinoazine Derivatives with Simple Nucleophiles, in Advances in Heterocyclic Chemistry, A. R. Katritzky, ed, Academic Press, New York, p 292.

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

[9] D. S. Noyce and M. D. Schiavelli, *J. Org. Chem.*, **33**, 845 (1968).

[10] K.-I. Tanji, T. Sakamoto and H. Yamanaka, *Chem. Pharm. Bull.*, **30**, 1865 (1982).

[11] M. J. Chapdelaine, P. J. Warwick and A. Shaw, *J. Org. Chem.*, **54**, 1218 (1989).

[12a] F. Marcuzzi, G. Modena, C. Paradisi, *J. Org. Chem.*, **50**, 4973 (1985); [b] A. D. Allen, Y. Chiang, A. J. Kresge and T. T. Tidwell, *J. Org. Chem.*, **47**, 775 (1982).

[13] H. Vorbrüggen and K. Krolikiewicz, *Angew. Chem.*, **21**, 724 (1976).

[14] J. I. Dickstein and S. I. Miller, Nucleophilic Attacks on Acetylenes, in The Chemistry of the Triple Bond, Vol **2**, S. Patai, ed, John Wiley and Sons, New York, 1978, Chapter 19.

[15] P. F. Hudrlik and A. M. Hudrlik in The Chemistry of the Carbon-Carbon Triple Bond, Part **1**, S. Patai, ed, John Wiley and Sons, New York, 1978, Chapter 7.

[16] 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.

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