A Chemical Model for the Fragmentation Reaction in Thymidylate Synthase Catalysis. Synthesis and Evaluation of a 5-Methylene-1-(1,2,3,4-tetrahydroquinolyl)-6-allyluridine

John R. Kagel,*,†,‡ Binghe Wang,§ and Mathias P. Mertes⊥

Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66045

Received October 16, 1992

Compounds **5** and **6** were synthesized as models to investigate the reactivity of proposed intermediate **2** in thymidylate synthase (TS) catalysis as it fragments to form dTMP. The mechanism of the fragmentation (homolytic or heterolytic) of model **6** was determined via subsequent interaction of the fragmented center with the C6 allyl substituent. The results were consistent with an ionic fragmentation of **6**, followed by loss of an allylic proton, and subsequent thermal electrocyclic or Diels–Alder reactions of the resulting trienes **13** and **14**, respectively. Independent generation of radicals analogous to that produced from a radical fragmentation of model **6** did not result in formation of trienes **13** and **14**.

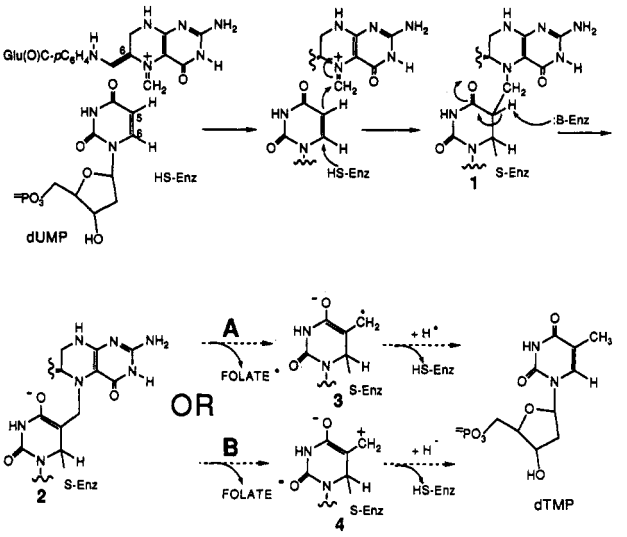
Introduction

Thymidylate synthase (TS, E.C. 2.1.1.45)[1] catalyzes the terminal step in the conversion of deoxyuridine 5′-monophosphate (dUMP) to thymidine 5′-monophosphate (dTMP) in the sole pathway for the de novo biosynthesis of one of the building blocks of DNA.[2] Due to this critical function, TS has received considerable attention as a target for anticancer agents (e.g., 5-fluorouracil).[3] Although many features of TS catalysis have been elucidated,[4] it is important to characterize a pathway in this enzymatic mechanism that remains in question. Such information might be of use in the development of new, or more effective, chemotherapeutic agents targeting TS.

It is established that addition of an enzyme sulfhydryl group to C6 of dUMP activates C5 for an attack on the cofactor, (6*R*)-5,10-methylenetetrahydrofolate, to form the ternary complex **1** (Scheme I).[5] Subsequent conversion of **1** to dTMP requires (1) removal of the C5 proton, affording proposed intermediate **2**,[4b] (2) fragmentation of **2** at the C–N bond of the methylene bridge, (3) stereospecific reduction of the bridging methylene carbon by a hydrogen from the cofactor,[2] and (4) reversal of the sulfhydryl addition.

Currently, the mechanism by which the proposed intermediate **2** fragments and is reduced to afford dTMP, remains to be determined. There is evidence supporting pathways A or B that produce radical[6] or ionic[7] intermediates **3** or **4**, respectively. Our interest was to determine which intermediate is produced in vivo from **2**. However, because proposed intermediate **2** is unstable and has not been isolated, in this preliminary effort we utilized the fragmentation of more stable model compounds (to facilitate their handling) as a means to study the fragmentation of **2**.

Scheme I



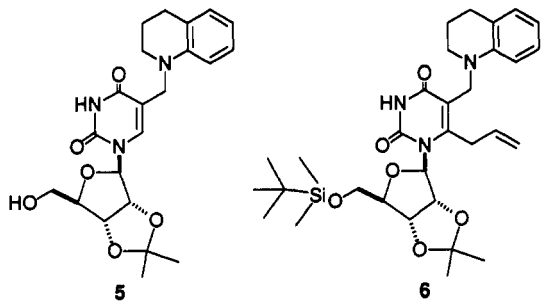
Design of the Chemical Models. The chemical models **5** and **6** were selected because they have certain important features in common with proposed intermediate **2**. The models (**5** and **6**) and **2** contain uridine with an sp^2-hybridized carbon at C5 joined through a methylene bridge to a tertiary nitrogen substituted with aromatic and cyclic aliphatic groups.

Because 6-substituted uridines (e.g., 5,6-disubstituted **6**) are not substrates for TS,[8] an alternative method was required to effect fragmentation/reduction at the C5-methylene bridge, analogous to the TS enzymatic reaction. We selected pyrolysis as the means to fragment model **6** because it has been used to fragment 5-substituted analogs

* Address correspondence to this author.
† Present address: Department of Pharmaceutical Chemistry, University of Kansas.
‡ Taken in part from the Ph.D. dissertation of J.R.K., University of Kansas, 1989.
§ Present address: Department of Chemistry, University of Arizona.
⊥ Deceased April 6, 1989.

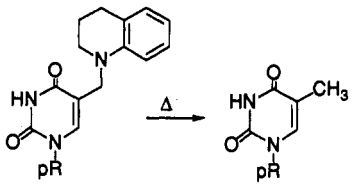
(1) Montfort, W. R.; Perry, K. M.; Fauman, E. B.; Finer-Moore, J. S.; Maley, G. F.; Hardy, L. W.; Maley, F.; Stroud, R. M. *Biochemistry* **1990**, *29*, 6964.
(2) Friedkin, M. *Ann. Rev. Biochem.* **1963**, *32*, 185.
(3) Cisneros, R. J.; Silks, L. A.; Dunlap, R. B. *Drugs Future* **1988**, *13*, 859.
(4) (a) Finer-Moore, J. S.; Montfort, W. R.; Stroud, R. M. *Biochemistry* **1990**, *29*, 6977. (b) Pogolotti, A. L., Jr.; Santi, D. V. In *Bioorganic Chemistry*; van Tamelen, E. E., Ed.; Academic Press: New York, 1977; Vol. I, pp 277–311.
(5) Moore, M. A.; Ahmed, F.; Dunlap, R. B. *Biochemistry* **1986**, *25*, 3311.
(6) Slieker, L. J.; Benkovic, S. J. *J. Am. Chem. Soc.* **1984**, *106*, 1833.
(7) (a) Heidelberger, C.; Danenberg, P. V.; Moran, R. G. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1983**, *54*, 57. (b) Mathews, D. A.; Villafranca, J. E.; Janson, C. A.; Smith, W. W.; Welsh, K.; Freer, S. *J. Mol. Biol.* **1990**, *214*, 937. For recent studies of N-3-substituted models, see: van der Meij, P. F. C.; Pandit, U. K. *Tetrahedron Lett.* **1988**, *29*, 5445.
(8) Kapuler, A. M.; Reich, E. *Biochemistry* **1971**, *10*, 4050.

0022-3263/93/1958-2738$04.00/0



5 6

in other TS catalysis studies,[9] such as 5-substituted model **5**,[10a] to afford 5-methyluridine analog **7**.[10] This is similar



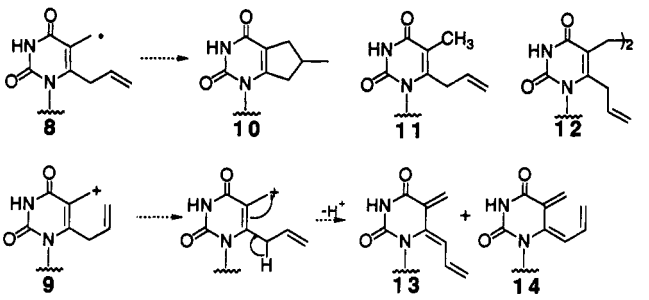
5 7

to the enzymatic fragmentation/reduction of proposed intermediate **2** to dTMP and indicates that pyrolytic fragmentation of 5-substituted N-methylenetetrahydroquinolines is suitable as a model of TS catalysis.

Methylene bridge fragmentation with loss of tetrahydroquinoline in 5,6-disubstituted model **6** will occur via either a radical or an ionic mechanism to produce unstable species **8** or **9**, respectively, which will undergo further reactions to afford more stable products (Scheme II). *The feature of these experiments that allows the fragmentation mechanism of 6 to be determined is that the final products produced from radical 8 are expected to differ from those produced from carbocation 9.* For example, radical **8** has a carbon radical located five atoms from a carbon–carbon double bond. Similar, though less complex, radicals (for example, a five-hexenyl radical) usually cyclize to afford five-membered rings[11] (e.g., **10**), although six-membered rings are formed when the radical center is substituted with sufficient radical-stabilizing groups.[12]

However, an alternative fate is possible for radical **8**. The 2-allylbenzyl radical (a conformationally restricted 5-hexa*dien*yl radical more analogous to radical **8**), is reported to undergo hydrogen atom abstraction without cyclization (formation of 2-allyltoluene was favored by 10.5 kcal/mol).[13] MINDO computational analysis revealed that 2-allylbenzyl radical cyclization required rotation of the radical orbital to a position approximately parallel to the aromatic ring, an unfavorable process because it results in a loss of radical stabilization energy.[13] By analogy, homolytic fragmentation of **6** to produce radical **8** might then be expected to result in hydrogen atom abstraction (from tetrahydroquinoline) to form the 5-methyl-6-allyl derivative **11**, rather than cyclized **10**. Alternatively, radical–radical coupling of **8**, affording dimer **12**, is also possible.

Scheme II



8 10 11 12

9 13 14

The product of an ionic fragmentation of 5,6-disubstituted **6** is carbocation **9** (Scheme II). Loss of the doubly-activated allylic proton adjacent to C6 is expected,[14] forming the cis,cis or cis,trans hexatrienes **13** or **14**. Trienes **13** or **14** may not be stable, and additional reactions analogous to those of the unsubstituted parent 1,3,5-hexatriene[15] might be anticipated.

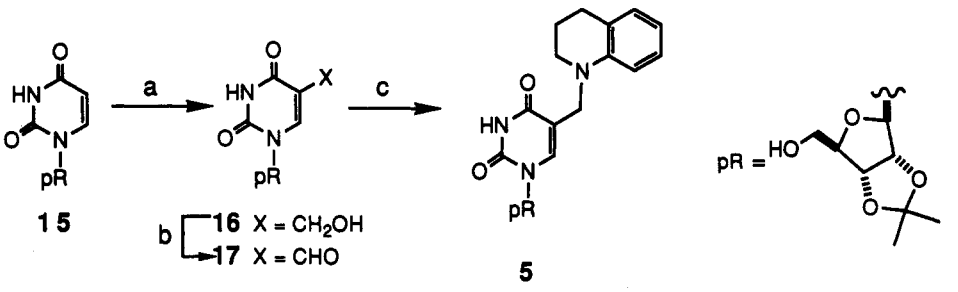
Results and Discussion

Synthesis of 5-Substituted or 5,6-Disubstituted Models 5 and 6. 5-Substituted model **5** was prepared (Scheme III) from 2′,3′-*O*-isopropylidineuridine (**15**) (pR = 2′,3′-*O*-isopropylidine-β-D-ribofuranosyl)[16] using reaction with formaldehyde and base at C5 to produce alcohol **16**.[17] Oxidation[17] afforded aldehyde **17**, which was converted directly to model **5** via a reductive amination. Preliminary studies using standard reductive amination conditions[18,19] (methanol, optimal pH, 72 h) resulted in poor yields (ca. 25%). However, the addition of 70 equiv of anhydrous zinc chloride (used stoichiometrically to facilitate reductive aminations of aldehydes[20]) to the reaction of aldehyde **17** in methanol resulted in a conversion (84%, 36 h) to C5-substituted model **5**.

In our early efforts to synthesize a 5,6-disubstituted uridine analog (e.g., **6**), the strategy of two sequential intermolecular reactions was examined as C5- or C6-monosubstituted products were prepared. Either propyl bromide/LDA was used to alkylate acetonide **15** at C6[21] or formaldehyde/base was reacted with C5 of uracil[22] or acetonide **15**,[17] and the resulting benzylic-like alcohol was then protected.[23] However, none of the C5- or C6-monosubstituted compounds could be converted to a corresponding 5,6-disubstituted product; apparently, monosubstitution at C5 or C6 precluded subsequent *inter*molecular reaction at the adjacent unsubstituted center. The 5,6-disubstituted **6** was obtained using an alternative strategy via acetal **18**[24] (Scheme IV), which was prepared

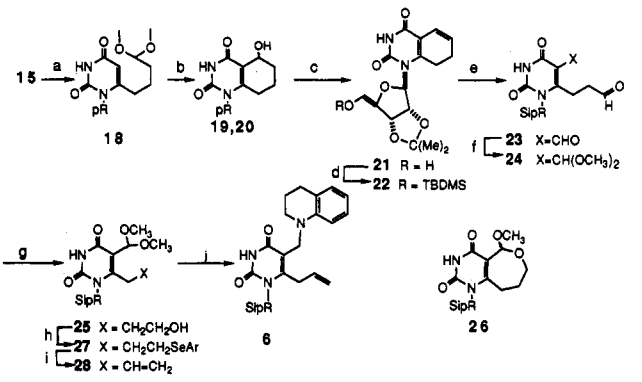
(9) Wilson, R. S.; Mertes, M. P. *Biochemistry* **1973**, *12*, 2879.
(10) (a) Wang, B.; Kagel, J. R.; Mertes, M. P., manuscript in preparation. (b) Jones, S. S.; Reese, C. B.; Ubasawa, A. *Synthesis* **1982**, 259.
(11) (a) Beckwith, A. L. J.; Ingold, K. U. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press: New York, 1980; Chapter 4. (b) Giese, B. *Radicals in Organic Synthesis: Formation of Carbon–Carbon Bonds*; Pergamon Press: New York, 1986; Chapter 4.
(12) (a) Julia, M. *Acc. Chem. Res.* **1971**, *4*, 386. (b) Julia, M. *Pure Appl. Chem.* **1974**, *40*, 553.
(13) Franz, J. A.; Suleman, N. K.; Alnajjar, M. S. *J. Org. Chem.* **1986**, *51*, 19 and references cited therein.
(14) March, J. *Advanced Organic Chemistry*, 3rd ed.; John Wiley: New York, 1985; p 150.
(15) Gajewski, J. J. *Hydrocarbon Thermal Isomerizations*; Academic Press: New York, 1981; Chapter 6.
(16) Levene, P. A.; Tipson, R. S. *J. Biol. Chem.* **1934**, *106*, 113.
(17) Armstrong, V. W.; Witzel, G.; Eckstein, F. In *Nucleic Acid Chemistry*; Townsend, L. B., Ed.; John Wiley and Sons: New York, 1986; Part 3, pp 65–69.
(18) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.
(19) Lane, C. F. *Synthesis* **1975**, 135.
(20) Kim, S.; Oh, C. H.; Ahn, K. H.; Kim, Y. J. *J. Org. Chem.* **1985**, *50*, 1927.
(21) Tanaka, H.; Nasu, I.; Miyasaka, T. *Tetrahedron Lett.* **1979**, *49*, 4755.
(22) Skinner, W. A.; Schelstraete, G. M.; Baker, B. R. *J. Org. Chem.* **1960**, *25*, 149.
(23) Brossmer, R.; Rohm, E. *Z. Physiol. Chem.* **1967**, *348*, 1431.
(24) Wang, B.; Kagel, J. R.; Rao, T. S.; Mertes, M. P. *Tetrahedron Lett.* **1989**, *30*, 7005.

Scheme III[a]



[a] Key: (a) ref 17; (b) ref 17; (c) tetrahydroquinoline, $Na(CN)BH_3$, $ZnCl_2$ (70 equiv), 36 h (84%).

Scheme IV[a]



[a] Key: (a) (i) LDA, (ii) $Br(CH_2)_3CH(OMe)_2$, −78 °C (80%); (b) 5% TFA (74%, 7%); (c) *p*-TsOH (82%); (d) TBDMS-Cl (93%); (e) O_3; Me_2S; (f) (i) SiO_2/MeOH, (ii) NH_4Cl/MeOH (88%); (g) $NaBH_4$ (0 °C → rt); NH_4Cl (0 °C → rt) (91%); (h) *o*-NO_2PhSeCN (86%); (i) H_2O_2 (quantitative); (j) tetrahydroquinoline, $Na(CN)BH_3$, $ZnCl_2$ (800 eq), 12 h (94%).

(79%) by alkylation of acetonide **15** with 4-bromobutanal dimethyl acetal.[25,26]

Treatment of acetal **18** with trifluoroacetic acid/acetone/water (5:85:10) resulted in a novel *intra*molecular cyclization to afford diastereomeric alcohols **19** and **20** (1:2, 74%).[24] The mixture of alcohols **19** and **20** was dehydrated to olefin **21**[27] (82%) by using *p*-TsOH.

After the C5′ hydroxyl group of olefin **21** was protected as its TBDMS ether[29] (**22**, 93%), ozone[30] was used to cleave the recently formed double bond to produce dialdehyde **23**.[31] During purification of dialdehyde **23** via radial chromatography (silica gel, MeOH), the silica catalyzed conversion of the benzylic-like aldehyde of **23** to a dimethyl acetal in **24**. The mixture of mono- and dialdehydes **24** and **23** was virtually impossible to separate, and so **24** and **23** were purified as a mixture. Residual dialdehyde **23** in the mixture after this chromatography was converted to dimethyl acetal **24** by treatment with ammonium chloride (catalytic) in methanol (88% yield of **24** from **22**).

Reduction of monoaldehyde **24** occurred in good yield (91%) to afford alcohol **25**, when care was taken to avoid cyclization affording **26**. Selenide formation[32] in the side chain of alcohol **25** (**27**, 86%) and oxidative elimination[33] produced the allyl group of acetal **28** (quantitative). Direct reductive amination of acetal **28** gave the 5,6-disubstituted target **6** (overnight, 94%), in a reaction where possible decomposition of **6** was minimized by increasing the reaction rate for its formation (relative to the reaction forming 5-substituted **5**, Scheme III) by using a considerable excess (ca. 800 equiv) of zinc chloride.

Pyrolytic Fragmentation of 5,6-Substituted Model 6. The pyrolysis of 5,6-disubstituted **6** resulted in the loss of tetrahydroquinoline. The mechanism of this fragmentation was determined based on the products formed from the interaction between the allyl side chain and the site of fragmentation. Minimum temperatures for the decomposition of **6** after heating for 10 min were as follows: 150–160 °C (neat), 150–155 °C (in polar, protic *n*-butanol), and 170–175 °C (in nonpolar, aprotic toluene).

HPLC analysis revealed that all three pyrolysis conditions produced the same seven-component (**A–G**) reaction profile (Table I). Extending the pyrolyses to 25 min did not reveal any new component. Because 2′,3′-*O*-isopropylidine-5′-(*tert*-butyldimethylsilyl)uridine (SipR = 2′,3′-*O*-isopropylidine-5′-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl) was stable at the pyrolysis conditions used, products formed from the pyrolysis of 5,6-disubstituted **6** must be due to features involving the tetrahydroquinoline and/or allyl substituents.

The identities of **A–C** were established as tetrahydroquinoline, quinoline, and model **6**, respectively, by NMR analysis and confirmed by coinjection of authentic standards. Component **D**, a shoulder on peak **C** and present only immediately (30–45 min) after pyrolysis, was unstable and decomposed to produce two new minor later eluting components that were not characterized further. Components **E**, **F**, and **G** were each stable at 175 °C. NMR analysis indicated that 5,6-disubstituted **6** (MW 583) lost tetrahydroquinoline (MW 133) in forming **E–G**. Mass spectral analysis demonstrated that component **E** was a monomeric product (*m/z* 450) and components **F** and **G** were dimers (*m/z* 900).

Homonuclear decoupling and carbon multiplicity analysis of monomer **E** were used to identify an intact SipR uridine system, with a $-CH_2CH_2CH{=}CH-$ moiety attached at C5 and C6. Component **E** was assigned as 5,6-dihydroquinazoline-2,4-dione (**29**, Scheme V), as the

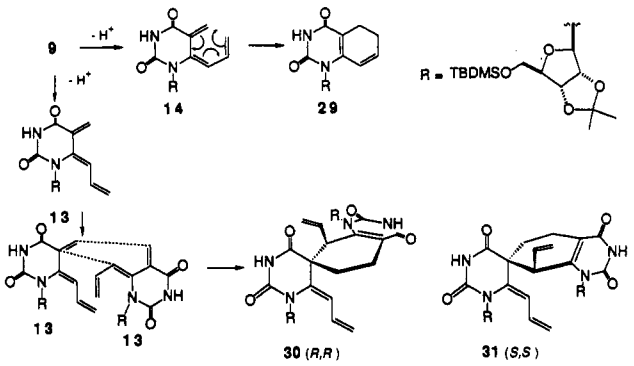
(25) Little, R. D.; Verhe, R.; Monte, W. T.; Nugent, S.; Dawson, J. R. *J. Org. Chem.* **1982**, *47*, 362.
(26) Peterson, J. S.; Toteberg-Kaulen, S.; Rapoport, H. *J. Org. Chem.* **1984**, *49*, 2948.
(27) It was important to carry **21** forward in the synthesis without delay, as it underwent gradual air oxidation to afford the aromatized quinazoline-2,4-dione analog. The ease of this oxidation was verified by using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone to form the same aromatized product. Deprotection of the acetonide afforded 1-β-D-ribofuranosylquinazoline-2,4-dione.[28]
(28) Stout, M. G.; Robins, R. K. *J. Org. Chem.* **1968**, *33*, 1219.
(29) Corey, E. J.; Venkateswarlu, A. *J. Am. Chem. Soc.* **1972**, *94*, 6190.
(30) Pappas, J. J.; Keaveney, W. P. *Tetrahedron Lett.* **1966**, 4273.
(31) Limited attempts to monitor this reaction via ozone-sensitive dyes were not successful. See: Veysoglu, T.; Mitscher, L. A.; Swayze, J. K. *Synthesis* **1980**, *41*, 807.
(32) Grieco, P. A.; Gilman, S.; Nishizawa, M. *J. Org. Chem.* **1976**, *41*, 1485.
(33) Grieco, P. A.; Nishizawa, M.; Ogure, T.; Burke, S. D.; Marinovic, N. *J. Am. Chem. Soc.* **1977**, *99*, 5773.

Table I. Reaction Profiles of Pyrolysis of 5,6-Disubstituted Model 6

peak	time[a]	identificn	% conversn					
			neat		*n*-butanol		toluene	
			10′	25′	10′	25′	10′	25′
A	3.0	tetrahydroquinoline						
B	7.3	quinoline						
C	9.3	6	84[b]	67	74	35	79	54
D	9.8	unstable						
E (29)	17.5	monomer	5	14	10	31	8	12
F (30)	31.6	dimer	5	11	10	23	8	22
G (31)	33.2	dimer	3	6	6	10	5	12
uncharacterized			3	3		1		

[a] Retention time (min), normal-phase HPLC. [b] Contribution of C plus D, assuming equal extinction coefficients at 270 nm.

Scheme V



alternative, **22** (prepared as a precursor to **6**), was not component **E**.

Identification of the dimers **F** and **G** ($C_{44}H_{68}N_4O_{12}Si_2$) was not as straightforward. A doubling of most, but not all, NMR signals for **F** and **G** suggested that each was an unsymmetrical molecule. COSY NMR analysis of dimers **F** and **G** demonstrated that each dimer had two intact ribose sugar rings with TBDMS and acetonide groups (accounting for 54 protons) present at their characteristic positions as observed for 5,6-disubstituted **6** and its precursors. Two protons for each dimer were exchanged readily upon treatment with D_2O, and each was assigned as an N–H.

Four of the remaining 12 protons were aliphatic, coupled only with each other. Carbon multiplicity (DEPT)[34] for dimer **F** indicated that these must be a $-CH_2CH_2-$ unit. Eight unassigned protons and 10 carbons were present as two $=CH_2$, three $=CH-$, one aliphatic CH, and four quaternary carbons. Although phase sensitive double quantum filtered (DQF) COSY[35] analysis indicated that these eight protons were contiguous, examination of all combinations of these groups showed that this was not possible.

The discrepancy was due to two overlapping multiplets whose couplings were not resolved completely by the DQF COSY, so that erroneous correlations were indicated. The only moieties consistent with the 2D and DEPT data were $C{=}CHCH{=}CH_2$, $(C)_2CHCH{=}CH_2$ (Table II), and one quaternary carbon. It was reasonable that these functional groups identified from dimer **F** were assembled as **30** (Scheme V); this structure was also supported by its MS fragmentation pattern.

Dimer **G** showed HPLC, mass spectral, UV, and NMR characteristics similar, or identical, to those of dimer **F**. Particularly informative were the DQF COSY results, indicating signals for two intact ribose sugar rings with TBDMS and acetonide groups, and for the nonsugar moieties comparable to those for dimer **F** (Table II). The nonsugar moiety of dimer **G** was assigned as the enantiomer of the nonsugar moiety of dimer **F**, and **G** was assigned as **31**. The rationale for assigning the major dimer as **30** is described below.

The most likely mechanism for formation of monomer **29** and dimers **30** and **31** involves trienes **13** and **14**, the products expected from an ionic fragmentation of 5,6-disubstituted **6**. Electrocyclic reaction of triene **14**, under the thermal conditions of the pyrolysis, can account for production of monomer **29**, whereas Diels–Alder reaction (DAR)[36a] dimerization of triene **13** will produce **30** and **31**. Because cis substituents on dienes hinder DAR, the cis,cis triene **14** is not expected to participate in dimer formation.[37]

Analogous to the DAR resulting in a dimerization of 1,3,5-hexatriene, the DAR of **13** involve a terminal double bond as the dieneophile in a [4 + 2], rather than [6 + 4], cycloaddition to produce the para-substituted adduct.[38] The double bond serving as the diene was locked in a cis orientation, a feature favorable for DAR.[36a] Selection of the exocyclic methylene group as the dieneophile may be due to the adjacent electron-withdrawing substituent (C=O).

Assignment of **30** and **31** as the major and minor products, respectively, of DAR dimerization of triene **13** was based on Dreiding-type and CPK models for the most favorable transition state for these reactions. Assuming transition states with (1) an endo orientation between trienes, and (2) a more favorable s-syn conformation of C6-substituted uridines along the glycoside bond,[39] the modeling indicated a severe steric interaction as the sugar moieties approached each other. Based upon these considerations, the least hindered (most favorable) transition state was assigned as **30A**, producing the major dimer product **30**; the alternative transition state (**31A**) was assigned to the minor dimer product **31**.

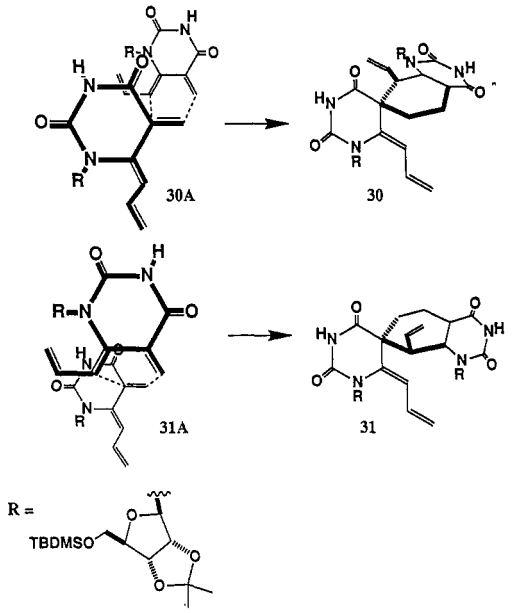
Finally, the approximately 5:1 ratio of dimers to monomer reflects a 10:1 ratio of cis,trans triene/cis,cis triene (**13** to **14**) produced by the pyrolysis. The preferential formation of cis,trans triene **13** may reflect the less

(34) Benn, R.; Gunther, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 350.
(35) (a) Marion, D.; Wuthrich, K. *Biochem. Biophys. Res. Commun.* **1983**, *113*, 967. (b) Derome, A. E. *Modern NMR Techniques for Chemistry Research*; Pergamon Press: Oxford, 1987; Chapter 8.
(36) (a) Woodward, R. B.; Hoffmann, R. *Angew. Chem., Int. Ed. Engl.* **1969**, *8*, 781. (b) Havinga, E.; Schlatmann, J. L. M. A. *Tetrahedron* **1961**, *16*, 146.
(37) Fleming, I. *Frontier Orbitals and Organic Chemical Reactions*; John Wiley: London, 1976; pp 165–176.
(38) Stewart, C. A., Jr. *J. Org. Chem.* **1963**, *28*, 3320.
(39) (a) Schweizer, M. P.; Banta, E. B.; Robins, R. K. *J. Am. Chem. Soc.* **1973**, *95*, 3770. (b) Wang, B.; Mertes, M. P.; Mertes, K. B.; Takusagawa, F. *Tetrahedron Lett.* **1990**, *31*, 4273.

Table II. Comparison of ^{1}H-NMR Assignments for the Nonsugar Signals of Dimer F (30) and Dimer G (31)

structure	δ[a]/multiplicity[b]/coupling constant, Hz							
	30				31			
—C=CHCH=CH$_2$	5.76[c]	7.10	5.39	5.35	5.80	6.65	5.40	5.38
	m	m	d	d	m	m	d	d
			17.8	10.1			17.4	9.7
—CHCH=CH$_2$	3.74	5.74	5.16	5.49	3.71	5.82	5.13	5.47
	m	m	d	d	m	m	d	d
			17.4	10.3			17.6	10.3
—CH$_2$CH$_2$—	3.16	2.57	2.08	1.85	3.19	2.59	2.08	1.87
	m	dd	m	dt	m	m	m	m
		18.2, 8.6		13.7, 9.5				
NH$_a$, NH$_b$	8.06	7.10			7.78	7.03		
	brs	m[d]			brs	brs		

[a] Chemical shift. [b] Key: brs, broad singlet; d, doublet; m, multiplet; t, triplet. [c] The order in the list corresponds to the order in the structure. [d] Overlapping signals.



hindered transition state for triene formation, in which the C6 allyl group is positioned away from the C5 substituent.

Independent Generation of Radical Species Similar to Radical 7. Additional support for the ionic fragmentation of 5,6-disubstituted model **6** to afford trienes **13** and **14** was obtained. The reactivity of the alternative intermediate (i.e., hydrogen atom abstraction without cyclization of radical **8**) was investigated by identifying the products formed from this species of radical when it was generated independently, using established chemical methods (Table III).

One method of generating a radical comparable to **8** utilized the greater reactivity of the benzylic-like hydroxyl group of diol **33** (prepared by deprotection of dimethyl acetal **28**, followed by aldehyde reduction)[40] with (thiocarbonyl)diimidazole to form unstable, water-sensitive compounds that did not survive chromatographic purification, presumably (thiocarbonyl)imidazole adduct **34**. Treatment of this mixture using radical deoxygenation[41-43] conditions via tri-*n*-butyltin hydride resulted in the formation of 5-methyl-6-allyl derivative **35** (not from diol **33**) and the starting diol **33**. Similar treatment[44] of the labile diiodo[45] derivative **36** afforded only the reduced uncyclized uridine analog **37**.

In limited studies, diiodo derivative **36** also was treated using conditions favoring[46] the cyclization of stabilized 5-hexenyl radicals ($h\nu$ irradiation of a 0.3 M solution containing 10% hexamethylditin or hexaphenylditin and then treatmnt with tri-*n*-butyltin hydride). Products (>10%) were isolated by HPLC and characterized by UV and/or mass spectral analysis. Reduced, uncyclized compound **37** was present, but no product had (1) a characteristic UV spectrum indicating the 5,6-dihydroquinazoline-2,4-dione moiety as in monomeric pyrolysis product **29** (component **E**) or (2) the *m/z* corresponding to a dimeric product, as in the polycyclic **30** and **31** (components **F** and **G**).

Thus, although our investigations were not exhaustive, they indicated that hydrogen atom abstraction to form 5-methyl-6-allyl analog **11**, without cyclization or loss of a hydrogen atom, is favored by radical **8**. For further support of an ionic fragmentation of 5,6-disubstituted model **6**, the uncyclized product **11** expected from a radical fragmentation/reduction of **6** was prepared independently (Table III); it was confirmed that this was not a product in the pyrolysis reactions of model **6**.

Conclusions. 5-Substituted model **5** undergoes thermally-induced fragmentation/reduction to produce dTMP analog **7**, a reaction similar to the TS enzyme-catalyzed conversion of **2** to dTMP. 5,6-Disubstituted model **6** undergoes thermally-induced fragmentation, but due to the C6 allyl side chain, additional reactions occur to produce monomer **29** and dimers **30** and **31**.

The most reasonable mechanism for formation of **29** was via a thermal electrocyclic reaction of triene **14** (Scheme V). However, because certain 5-hexenyl radicals cyclize to form six-membered rings, it was important to demonstrate that radical **8** did not contribute to formation of cyclized **29** (Table III). Formation of the dimers **30** and **31** from the pyrolysis of 5,6-disubstituted **6** indicated the presence of triene **13** (and therefore, presumably also **14**) and supports the ionic fragmentation mechanism.

The ionic mechanism for the fragmentation of 5,6-disubstituted **6**, with a polar transition state, was also consistent with the solvent effects noted, in which use of

(40) It was optimal not to isolate aldehyde **32** prior to its reduction, as isolation of **32** followed by its reduction resulted in a significant (ca. 50%) decrease in the overall yield of diol **33** produced from acetal **28**.
(41) Barton, D. H. R.; Motherwell, W. B.; Stange, A. *Synthesis* **1981**, 743.
(42) Barton, D. H. R., McCombie, S. W. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1574.
(43) For a review of radical deoxygenation reactions, see: Hartwig, W. *Tetrahedron* **1983**, *39*, 2609.
(44) Curran, D. P. *Synthesis* **1988**, 417.
(45) Landauer, S. R.; Rydon, H. N. *J. Chem. Soc. C* **1953**, 2224.
(46) Curran, D. P.; Chang, C. T. *J. Org. Chem.* **1989**, *54*, 3140.

Table III. Chemical Generation of C5-Methylene Radicals in 6-Allyluridine Analogs

compd	X	Y	treatment	product (yield, %)	X′	Y′
28	OTBDMS	$CH(OMe)_2$	$AcOH/H_2O$	**32** (not isolated)	OH	CHO
32	OH	CHO	$NaBH_4$	**33** (92%)[a]	OH	CH_2OH
33	OH	CH_2OH	*n*-Bu_3SnH/AIBN 80 °C	no reaction		
33	OH	CH_2OH	$C(=S)(Imid)_2$	**34** (proposed, not isolated)	OH	$CH_2OC(=S)$-Imid
34	OH	$CH_2OC(=S)$-Imid	*n*-Bu_3SnH/AIBN	**35** (8)	OH	CH_3
			80 °C, then SiO_2	**33** (92)[b]	OH	CH_2OH
33	OH	CH_2OH	$CH_3P(OPh)_3I$	**36** (proposed,[c] not isolated)	I	I
36	I	I	2 equiv of *n*-Bu_3SnH/AIBN/*hν*	**37** (62) + 7 other minor components	H	CH_3
36	I	I	7 equiv of *n*-Bu_3SnH/AIBN/*hν*	**37** (100)	H	CH_3
35	OH	CH_3	TBDMS-Cl/DMAP	**11** (100)[d]	OTBDMS	CH_2OH

[a] Yield from **28**. [b] Based on isolated **35**. [c] Quantitative, based on conversion of **36** to **37**. [d] Estimated yield by TLC analysis.

a polar solvent resulted in the most facile fragmentation (Table I). Finally, the major product expected from a radical fragmentation of 5,6-disubstituted **6** (reduced uncyclized **11**) was prepared independently and demonstrated not to be present after the pyrolysis.

Although one must be cautious in extrapolating an ionic fragmentation from the model used here to the enzymatic mechanism of TS catalysis, it should be noted that our results are consistent with the mechanism proposed by other investigators based upon results involving stabilized analogs of the ternary complex.[7b]

Experimental Section

Preparative centifugal thin-layer (radial) chromatography was performed on a Harrison Model 7924 Chromatotron using Merck silica gel 60 PF-254 containing $CaSO_4 \cdot 1/2\ H_2O$ binder. Flash chromatography refers to the method of Still et al.[47] Solvent systems for thin-layer chromatography are noted as R_f (A, B, or C) where A indicates solvent system 5% ethanol/95% methylene chloride, B indicates 10% ethanol/90% methylene chloride, and C indicates 25% hexane/75% ethyl acetate. High-performance liquid chromatography (HPLC) was performed on a Walters Delta Prep 3000 fitted with a normal-phase μPorasil column (Waters, 3.9 mm × 15 cm) using a 1 mL/min flow rate of hexane/ethyl acetate (4:1) for 20 min and then a linear gradient to ethyl acetate at 40 min and ethyl acetate thereafter. Photochemical reactions were performed in Pyrex vessels irradiated with a 275-W Hanovia mercury lamp.

HPLC solvents were Optima grade (Fisher). Benzene and tetrahydroquinoline were distilled from calcium hydride. Butanol (Baker, reagent grade), tri-*n*-butylphosphine, and tri-*n*-butyltin hydride were distilled and stored under argon. Toluene (anhydrous, Aldrich, Spectrophotometric grade) and (*o*-nitrophenyl)-seleno cyanate (Fluka) were purchased. Solvents for pyrolysis or tin reactions were degassed immediately prior to use by bubbling argon through them for 45 min. Azobisisobutyronitrile (AIBN, Chemical Dynamics Corp.) was crystallized from acetone. Sodium cyanoborohydride was purified by pyrolysis of its dioxane complex and stored under argon. Anhydrous zinc chloride was fused and cooled under vacuum immediately before use. Methyltriphenoxyphosphonium iodide was triturated with dry tetrahydrofuran until the washings were colorless and then dried under vacuum. Ozone was generated from a Welsbach T-23 Ozonator.

1,2,3,4-Tetrahydro-1-(2′,3′-*O*-isopropylidinethymidyl)-quinoline (5). 5-Formyl-2′,3′-*O*-isopropylidineuridine (**17**) (350 mg, 1.1 mM) was dissolved in 40 mL of MeOH under argon. 1,2,3,4-Tetrahydroquinoline (2.5 mL, 19.9 mM), anhydrous zinc chloride (9.8 g, 71.4 mM), and sodium cyanoborohydride (120 mg, 1.9 mM) were added. After 36 h the solvent was removed, 25 mL of water was added, and the aqueous layer was extracted with hexane (3 × 25 mL). The organic layers were pooled, extracted with 15 mL of water, and concentrated. Radial chromatography (1 mm SiO_2, eluant: methylene chloride/MeOH gradient) afforded 370 mg (440 mg theor, 84%) of **5** as a foam: ^1H NMR (300 MHz) δ 8.79 (bs, 1 H, NH), 7.23 (s, 1 H, C6-H), 7.01 (m, 2 H, Ar), 6.62 (d, J = 6 Hz, 1 H, Ar), 6.39 (d, J = 7.5 Hz, 1 H, Ar), 5.73 (d, J = 2.2 Hz, 1 H, C1′-H), 4.76 (m, 2 H, C2′-H overlapping C3′-H), 4.21 (m, 3 H, C4′-H overlapping C5-CH_2), 3.36 (t, J = 5.5 Hz, 2 H, CH_2CH_2NAr), 2.81 (t, J = 6.2 Hz, 2 H, CH_2Ar), 1.99 (m, 2 H, CH_2CH_2Ar), 1.55 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3); ^{13}C NMR (75 MHz) δ 162.94 (C-4), 150.12 (C-2), 144.75 (N(*C*Ar)), 137.43 (C-6), 129.25 (Ar), 127.37 (Ar), 122.31 (CH_2-(*C*Ar)), 116.53 (Ar), 114.03 ($C(CH_3)_2$), 110.66 (Ar), 109.80 (C-5), 94.19 (C-1′), 86.24 (C-4′), 84.38 (C-2′), 80.59 (C-3′), 62.93 (C-5′), 50.19 (C5-CH_2), 48.18 (CH_2CH_2N), 27.96 (CH_2Ar), 27.24 (CH_3), 25.25 (CH_3), 22.45 (CH_2CH_2N); R_f(B) = 0.31. Anal. Calcd for $C_{22}H_{27}N_3O_6$: C, 61.52; H, 6.34; N, 9.78. Found: C, 61.20; H, 6.48; N, 9.55.

1-(2′,3′-*O*-Isopropylidineurid-6-yl)-4-butyraldehyde Dimethyl Acetal (18). A solution of **15** (5.0 g, 17.5 mM) in 200 mL of THF was added slowly (45 min) to a solution of LDA (87.8 mM) in 20 mL of THF at −78 °C. After the solution was stirred for an additional 1 h, 4-bromobutyraldehyde dimethyl acetal (10.5 g, 53 mM) was added, and the solution was stirred at −78 °C for 90 h. Acetic acid (6 mL) was added, the reaction was warmed to room temperature, and the volatiles were removed under vacuum. The residue was dissolved in 60 mL of water and extracted with chloroform (7 × 100 mL). The organic layers were pooled, dried over sodium sulfate, and purified via flash chromatography (SiO_2, methylene chloride/MeOH (0–2%) gradient) to afford 5.54 g (79%, 7.01 g theor) of **18** as a white solid, R_f(A) = 0.51. Spectral characterization matched those reported.[24]

1-(2′,3′-*O*-Isopropylidine-β-D-ribofuranosyl)-5,6,7,8-tetrahydro-5α(or β)-hydroxyquinazoline-2,4-dione (19 or 20). Acetal **18** (5.0 g, 12.55 mM) in 485 mL of anhydrous acetone and 73 mL of TFA/water (1:2) was stirred overnight. The mixture was neutralized with sodium bicarbonate (30 g, 1.1 equiv) and filtered using acetone washes (4 × 20 mL). The filtrate and washes were pooled, concentrated, and dried under high vacuum (complete removal of water was necessary). The residue was triturated with THF/hexane (3:1) and filtered through a silica plug. Methylene chloride (20 mL) was added to the residue, followed by just enough methanol to dissolve all of the material. This was passed through a column eluted with methylene chloride/MeOH (0–4%) gradient). All of the organic fractions and washes containing products were pooled, concentrated, and purified by flash chromatography (SiO_2, eluant: methylene chloride/methanol (0–4%) gradient) to afford 3.2 g (4.34 g theor,

(47) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

74%) of a foam, **19** and **20**, with R_f(B) = 0.50 and 0.49, and spectral characteristics matching those reported.[24]

1-(2′,3′-*O*-Isopropylidine-β-D-ribofuranosyl)-7,8-dihydroquinazoline-2,4-dione (21). A mixture of the alcohols **19** and **20** (3.2 g, 9.0 mM), in 300 mL of acetone containing a few crystals of *p*-TSOH, was heated at reflux overnight while connected to a Dean-Stark trap filled with 4A molecular sieves. After cooling, the mixture was neutralized (sodium bicarbonate) and filtered, using acetone washes. The washes were pooled, concentrated, and dissolved in 50 mL of methylene chloride/water (1:1). The organic layer was removed, and the aqueous layer was washed (2 × 25 mL) with methylene chloride. The organic layers were pooled, concentrated, and purified by flash chromatography (SiO_2, eluant: methylene chloride/MeOH gradient) to afford 2.48 g (3.04 g theor, 82%) of **21** as a foam. Hexane added to diethyl ether/methylene chloride (10:1) solution of **21** afforded crystals: mp 168.5–169.5 °C; R_f(B) = 0.52.

1-(2′,3′-*O*-Isopropylidine-5′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)-7,8-dihydroquinazoline-2,4-dione (22). To **21** (2.0 g, 5.95 mM), in 100 mL of DMF under argon, were added triethylamine (1.10 mL, 7.93 mM), DMAP (0.24 g, 1.96 mM), and TBDMS chloride (1.0 g, 6.64 mM). The solution was stirred overnight, filtered, and washed with DMF (3 × 25 mL). Water (20 mL) was added to the filtrate, and it was stirred for 1 h. After solvent was removed, the residue was dissolved in hexane and purified by flash chromatography (SiO_2, eluant: hexane/methylene chloride (0–100%) gradient and then methylene chloride/MeOH (0–2%) gradient) to afford 2.5 g of **22** as a foam (2.68 g theor, 93%). Recrystallization (hexane) afforded clear crystals: mp 141.5–142.5 °C; R_f(A) = 0.48; ^{1}H NMR (500 MHz) δ 10.38 (bs, 1 H, NH), 6.49 (d, J = 9.7 Hz, 1 H, C5-H), 5.80 (bs, 1 H, C1′-H), 5.73 (td, J = 9.7, 4.5, 4.2 Hz, 1 H, C6-H), 5.20 (dd, J = 6.3, 0.7 Hz, 1 H, C2′-H), 4.82 (dd, J = 6.3, 4.5 Hz, 1 H, C3′-H), 4.13 (dbrt, J = 6.8, 4.8 Hz, 1 H, C4′-H), 3.79 (dd, J = 7.1, 5.0 Hz, 2 H, C5′-H), 2.77 (m, 2 H, C7-H), 2.38 (bs, 2 H, C8-H), 1.50 (s, 3 H, $C(CH_3)_2$), 1.30 (s, 3 H, $C(CH_3)_2$), 0.84 (s, 9 H, $C(CH_3)_3$), 0.01 (s, 6 H, CH_3Si); ^{13}C NMR (125 MHz) δ 162.29 (C-4), 150.04 (C-2), 148.72 (C-8a), 121.88 (C-6), 119.25 (C-5), 113.61 ($C(CH_3)_2$), 108.69 (C-4a), 90.66 (C-1′), 89.51 (C-4′), 84.19 (C-2′), 81.85 (C-3′), 64.11 (C-5′), 27.16 ($C(CH_3)_2$), 25.88 ($C(CH_3)_3$), 25.36 ($C(CH_3)_2$), 23.84 (C-8), 22.17 (C-7), 18.42 ($C(CH_3)_3$), −5.29 (($CH_3)_2Si$); UV (MeOH) λ_{max} = 311, 247 nm; λ_{min} = 273 nm. Anal. Calcd for $C_{22}H_{34}N_2O_6Si$: C, 58.64; H, 7.61; N, 6.22. Found: C, 58.25; H, 7.34; N, 6.08.

1-(2′,3′-*O*-Isopropylidine-5′-*O*-(*tert*-butyldimethylsilyl))-5-(dimethoxymethyl)-6-(3-oxopropyl)uridine (24). Ozone (10 mL/min generated at 60 volts) was bubbled slowly into a solution of **22** (378 mg, 0.84 mM) in 70 mL of methylene chloride stirred rapidly at −78 °C. When the first tint of purple color was noted in the reaction (16.5 min), the following were performed quickly: (1) the addition of ozone was stopped, (2) the solution was flushed with nitrogen, and (3) 6 mL (81.7 mM) of dimethyl sulfide was added. The solution was flushed with nitrogen for 30 min at −78 °C and for an additional 45 min with the cooling bath removed. Methanol (5 mL) was added and solvent removed under reduced pressure until approximately 5 mL of solvent remained. Purification by radial chromatography (2 mm SiO_2, eluant: methylene chloride/MeOH gradient) afforded a foam composed of dialdehyde **23** and dimethyl acetal **24**, with virtually identical R_f(A) = 0.42.

Ammonium chloride (0.1 g) was added to the mixture of **23** and **24** (totalling 0.84 mM) in 10 mL of MeOH under argon. After 2 h, until only one component was present (SiO_2 TLC, hexane/ethyl acetate = 1/1; cospotting was required to differentiate between the two compounds). The solvent was removed, and 100 mL of methylene chloride/water (1:1) was added to the residue. The organic layer was removed, and the aqueous layer was extracted with methylene chloride (2 × 25 mL). The organic layers were pooled, extracted with water (15 mL), and then concentrated to afford 391 mg (444 mg theor from **22**, 88%) of **24** as a foam.

1-(2′,3′-*O*-Isopropylidine-5′-*O*-(*tert*-butyldimethylsilyl))-5-(dimethoxymethyl)-6-(3-hydroxypropyl)uridine (25). Sodium borohydride (10 mg, 265 μM) was added to the dimethyl acetal **24** (29 mg, 55 μM) in 5 mL of MeOH at 0 °C. After 10 min at 0 °C and 20 min at room temperature, the temperature was lowered to 0 °C, and ammonium chloride (100 mg, 1.9 mM) was added. The solution was stirred for 10 min at 0 °C and 20 min at room temperature. The solvent was removed, and 10 mL of methylene chloride/water (1:1) was added to the residue. The organic layer was removed, and the aqueous layer was extracted with methylene chloride (2 × 5 mL). The organic layers were pooled, concentrated, and purified by radial chromatography (1 mm SiO_2, eluant: methylene chloride/MeOH gradient) to afford 26.5 mg theor, 91%) of **24** as a foam. R_f(C) = 0.55.

1-(2′,3′-*O*-Isopropylidine-5′-*O*-(*tert*-butyldimethylsilyl))-5-(dimethoxymethyl)-6-(3-((*o*-nitrophenyl)seleno)propyl)-uridine (27). THF (5 mL) was added to 871 mg of **25** (1.64 mM) and 447 mg (1.97 mM) of (*o*-nitrophenyl)seleno cyanate under argon. Tri-*n*-butylphosphine (0.53 mL, 2.1 mM) was added and the mixture stirred for 1 h. The solvent was removed, and the residue was purified by flash chromatography (SiO_2, eluant: methylene chloride/MeOH (0–2%) gradient) to afford 1.01 g (1.18 g theor, 86%) of a bright yellow foam. Due to facile air oxidation and elimination, accurate combustion and mass spectral analysis were not possible, R_f(C) = 0.55.

1-(2′,3′-*O*-Isopropylidine-5′-*O*-(*tert*-butyldimethylsilyl))-5-(dimethoxymethyl)-6-allyluridine (28). Hydrogen peroxide (0.90 mL of a 35% aqueous solution, 9.3 mM) was added slowly to selenide **27** (1.01 g, 1.41 mM) in 20 mL of THF at 0 °C. The mixture was warmed to room temperature and stirred overnight. The solvent was removed, and 250 mL of methylene chloride/saturated aqueous sodium bicarbonate (4:1) was added to the residue. The organic layer was removed, and the aqueous layer was washed with methylene chloride (2 × 50 mL). The organic layers were pooled and washed with 25 mL of water. Removal of the solvent afforded 765 mg (725 mg theor, quantitative) of **28** as a pale yellow foam, R_f(C) = 0.77.

1,2,3,4-Tetrahydro-1-(2′,3′-*O*-isopropylidine-5′-*O*-(*tert*-butyldimethylsilyl)-6-allylthymidyl)quinoline (6). Dimethyl acetal **28** (9.7 mg, 0.019 mM), 1,2,3,4-tetrahydroquinoline (0.05 mL, 0.40 mM), anhydrous zinc chloride (2 g, 14.7 mM), and sodium cyanoborohydride (20 mg, 0.32 mM) were dissolved in 2 mL of MeOH under argon. After the mixture was stirred overnight, solvent removal produced a residue that was dissolved in 20 mL of hexane/water (1/1). The organic layer was removed, and the aqueous layer was extracted with hexane (3 × 10 mL). The organic layers were pooled, extracted with 5 mL of water, and concentrated to afford a residue. This was purified by HPLC injections of 1-mg aliquots of the residue in chloroform (retention time = 9.3 min) to afford 10.1 mg (10.7 mg theor, 94%) of **6** as a film: R_f(A) = 0.65; ^{1}H-NMR (500 MHz, homonuclear decoupling) δ 7.07 (m, 1 H, Ar), 6.97 (d, J = 7.5 Hz, 1 H, Ar), 6.76 (d, J = 8 Hz, 1 H, Ar), 6.65 (t, J = 7.2 H, 1 H, Ar), 5.88 (m, 1 H, C*H*=CH_2), 5.72 (s, 1 H, C1′-H), 5.27 (d, J = 10.2 Hz, 1 H, CH=C*H*$_2$), 5.19 (d, J = 6 Hz, 1 H, C2′-H), 5.09 (d, J = 17.0 Hz, 1 H, CH=C*H*$_2$), 4.78 (dd, J = 6.3, 4.4 Hz, 1 H, C3′-H), 4.15 (m, 3 H, C2′-H overlapping on (C5-C*H*$_2$N), 3.82 (m, 2 H, C5′-H), 3.65 (dm, J = 17.1, 2.3 Hz, 1 H, C6-C*H*$_2$), 3.38 (dbd, J = 11.5, 6.0 Hz, 1 H, C6-C*H*$_2$), 3.03 (m, 2 H, CH_2CH_2N), 2.72 (m, 2 H, CH_2Ar), 1.87 (app quintet, J = 6.0 Hz, 2 H, CH_2CH_2N), 1.50 (s, 3 H, $C(CH_3)_2$), 1.31 (s, 3 H, $C(CH_3)_2$), 0.87 (s, 9 H, $C(CH_3)_3$), 0.03 (s, 6 H, $(CH_3)_2$-Si); ^{13}C NMR (125 MHz, DEPT) δ 163.57 (C-4), 153.65 (C-2), 150.57 (C-6), 146.12 (N(CAr)), 130.73 (*C*H=CH_2), 129.19 (Ar), 127.08 (Ar), 124.55 (Ar), 118.81 (CH=*C*H$_2$), 117.27 (Ar), 113.59 ($C(CH_3)_2$), 111.55 (Ar), 109.86 (C-5), 92.17 (C-1′), 89.65 (C-4′), 84.32 (C-2′), 82.05 (C-3′), 64.32 (C-5′), 46.60 (C5-CH_2), 44.12 (CH_2CH_2N), 27.94 (CH_2Ar), 27.21 ($C(CH_3)_2$), 25.91 ($C(CH_3)_3$), 25.38 ($C(CH_3)_2$), 22.24 (CH_2CH_2N), 18.45 ($C(CH_3)_3$), −5.20 (($CH_3)_2$-Si); UV (MeOH) λ_{max} = 262 nm (ε = 21 000), shld = 302 nm (ε = 3000), λ_{min} = 230 nm (ε = 7000); UV (H_2O) λ_{max} = 269 nm (ε = 17 500) shld = 313 nm (ε = 6500), λ_{min} = 241 nm (ε = 10 000); UV (0.1 N NaOH) λ_{max} = 261 nm (ε = 16 000), shld = 302 nm (ε = 3000), λ_{min} = 234 nm (ε = 10 000); UV (0.1 N HCl) λ_{max} = 269 nm (ε = 9500), λ_{min} = 235 nm (ε = 2500); EIHRMS *m/e* 583.3075 ($C_{31}H_{45}N_2O_6Si$ requires 583.3075). Anal. Calcd for $C_{31}H_{45}N_2O_6Si$: C, 63.78; H, 7.77; N, 7.20. Found: C, 63.83; H, 8.00; N, 6.98.

Pyrolysis of 5,6-Disubstituted Model 6. For the pyrolysis in solution, ca. 50 μg of **6** was placed in a Wheaton vial (1 mL) equipped with a magnetic stirrer and unlined plastic caps fitted with septa (Teflon-faced silicon for 20-mm seals, cut to fit the cap). Degassed butanol or toluene (0.4 mL) was added, and the

contents were capped under argon. The vials were immersed in oil baths at 150–155 or 170–175 °C for the butanol or toluene reactions, respectively. After 10 or 25 min, the vials were removed and cooled to rt, and solvent was removed via flushing with argon, followed by high vacuum. Chloroform (0.05 mL) was added, and 10-μL aliquots were analyzed by HPLC.

For the neat pyrolysis, the reaction vial was a 10-mL pear-shaped flask; the pyrolysis was conducted under a slight positive pressure of argon gas and at an oil bath temperature of 150–160 °C. The processing of these samples was as above, except for the removal of the pyrolysis solvent.

1-(2′,3′-*O*-Isopropylidine-5′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl)-5,6-dihydroquinazoline-2,4-dione (29). This compound was a product of the pyrolysis reaction of **6**, R_f(A) = 0.60. It was isolated using HPLC (retention time = 17.5 min): ^{1}H NMR (500 MHz, homonuclear decoupling) δ 6.53 (m, 2 H, C7-H and C8-H), 5.90 (d, J = 1.9 Hz, 1 H, C1′-H), 5.14 (dd, J = 6.6, 2.1 Hz, C2′-H), 4.84 (dd, J = 6.6, 4.4 Hz, 1 H, C3′-H), 4.10 (dt, J = 4.8, 5.9 Hz, 1 H, C4′-H), 3.83 (dd, J = 11.0, 4.7 Hz, 1 H, C5′-H_a), 3.73 (dd, J = 11.0, 6.1 Hz, 1 H, C5′-H_b), 2.58 (m, 1 H, C5-H_a), 2.50 (m, 1 H, C5-H_b), 2.25 (m, 2 H, C6-H_2), 1.53 (s, 3 H, C(CH_3)$_2$), 1.32 (s, 3 H, C(CH_3)$_2$), 0.87 (s, 9 H, C(CH_3)$_3$), 0.04 (s, 3 H, CH_3Si), 0.04 (s, 3 H, CH_3Si); ^{13}C NMR (125 MHz, off-resonance decoupled) δ 162.17 (C-4), 150.19 (C-2), 145.41 (C-8$_a$), 140.17 (C-8), 119.05 (C-7), 114.11 (*C*(CH_3)$_2$), 107.31 (C-4$_a$), 90.94 (C-1′), 88.21 (C-4′), 83.65 (C-2′), 81.12 (C-3′), 63.47 (C-5′), 27.21 (C(*C*H_3)$_2$), 25.95 (C(*C*H_3)$_3$), 25.41 (C(*C*H_3)$_2$), 21.79 (C-5), 18.48 (*C*(CH_3)$_2$), 18.28 (C-6), −5.27 (CH_3Si), −5.31 (CH_3Si); UV (MeOH) λ_{max} = 316 nm (ε = 14 000), λ_{min} = 266 nm; UV (H_2O) λ_{max} = 318 nm (ε = 12 500), λ_{min} = 268 nm; UV (0.1 N NaOH) λ_{max} = 317 nm (ε = 12 500), λ_{min} = 268 nm; UV (0.1 N HCl) λ_{max} = 318 nm (ε = 13 000), λ_{min} = 267 nm; EIHRMS m/e 450.2199 ($C_{22}H_{34}N_2O_6$Si requires 450.2186). Anal. Calcd for $C_{22}H_{34}N_2O_6$Si: C, 58.64; H, 7.61; N, 6.22. Found: C, 58.60; H, 7.38; N, 6.00.

The Major Dimer Product 30. This compound was a product of the pyrolysis of **6**, R_f(A) = 0.49. It was isolated using HPLC (retention time = 31.6 min): ^{1}H NMR (500 MHz; homonuclear decoupling; normal COSY; phase-sensitive double quantum filtered COSY; long-range COSY) δ 8.06 (bs, 1 H, NH_a), 7.10 (m, 2 H, NH_b and C=CHC*H*=CH_2), 5.76 and 5.74 (m, 2 H, C=C*H*CH=CH_2 and CCHC*H*=CH_2), 5.49 (d, J = 10.3 Hz, 1 H, CCHCH=CH_2), 5.48 (s, 1 H, C1′$_a$-H), 5.39 (d, J = 17.8 Hz, 1 H, C=CHCH=C*H*$_2$), 5.35 (d, J = 10.1 Hz, 1 H, C=CHCH=C*H*$_2$), 5.27 (d, J = 6.3 Hz, C2′$_a$-H), 5.16 (d, J = 17.4 Hz, 1 H, CCHCH=C*H*$_2$), 5.10 (d, J = 2.0 Hz, 1 H, C1′$_b$-H), 5.07 (d, J = 6.4 Hz, 1 H, C2′$_b$-H), 4.81 (dd, J = 6.2, 3.6 Hz, 1 H, C3′$_a$-H), 4.78 (dd, J = 6.4, 4.2 Hz, 1 H, C3′$_b$-H), 4.16 (dt, J = 4.2, 6.5 Hz, 1 H, C4′$_b$-H), 4.09 (dt, J = 3.5, 6.5, 1 H, C4′$_a$-H), 3.88 (d, J = 6.3 Hz, 2 H, C5′$_b$-H), 3.74 (bd, J = 6.4 Hz, 1 H, CC*H*CH=CH_2), 3.69 (d, J = 1.4 Hz, 1 H, C5′$_a$-H), 3.67 (d, J = 1.7 Hz, 1 H, C5′$_a$-H), 3.16 (m, 1 H, CH_2C*H*$_2$), 2.57 (dd, J = 18.2, 8.6 Hz, 1 H, CH_2C*H*$_2$), 2.08 (m, 1 H, C*H*$_2$CH$_2$), 1.85 (dt, J = 13.7, 9.5 Hz, C*H*$_2$CH$_2$), 1.51 (s, 3 H, C(C_aH_3)$_2$), 1.42 (s, 3 H, C(C_bH_3)$_2$), 1.33 (s, 3 H, C(C_aH_3)$_2$), 1.27 (s, 3 H, C(C_bH_3)$_2$), 0.88 (s, 9 H, C(C_aH_3)$_3$), 0.87 (s, 9 H, C(C_bH_3)$_3$), 0.06 (s, 6 H, (C_aH_3)$_2$Si), 0.04 (s, 3 H, C_bH_3Si), 0.03 (s, 3 H, C_bH_3Si); ^{13}C NMR (125 MHz; DEPT) δ 169.31 (C-4$_b$), 161.87 (C-4$_a$), 161.84 (C-2$_a$), 149.77 (C-2$_b$), 148.77 (C-6$_a$), 134.11 (*C*=CHCH=CH_2), 131.41 (C=C*H*CH=CH_2), 129.97 (CCH-*C*H=CH_2), 122.72 (C=CHCH=CH_2), 122.59 (C_2-CHCH=CH_2), 121.84 (C=*C*HCH=CH_2), 113.84 (*C*(CH_3)$_2$), 112.94 (*C*(CH_3)$_2$), 112.22 (C-5$_a$), 97.27 (C-1′$_a$), 91.25 (C-1′$_b$), 89.48 (C-4′$_a$), 88.05 (C-4′$_b$), 84.05 (C-2′$_a$), 83.47 (C-2′$_b$), 82.49 (C-3′$_a$), 82.21 (C-3′$_b$), 64.09 (C-5′$_a$), 63.73 (C-5′$_b$), 43.83 (C_2-CHCH=CH_2), 29.69 (CH_2C*H*$_2$), 27.21 (C(*C*H_3)$_2$), 27.19 (C(*C*H_3)$_2$), 25.93 (C(*C*H_3)$_3$), 25.88 (C(*C*H_3)$_3$), 25.58 (C(*C*H_3)$_2$), 25.23 (C(*C*H_3)$_2$), 20.40 (C-5$_b$), 20.17 (*C*H_2CH$_2$), 18.41 (*C*(CH_3)$_3$), 18.37 (*C*(CH_3)$_3$), −5.16 (CH_3Si), −5.19 (CH_3Si), −5.25 (CH_3Si), −5.30 (CH_3Si); UV (MeOH) λ_{max} 266 nm (ε 22 500), λ_{min} 236 nm; (H_2O) λ_{max} 268 nm (ε 22 500), λ_{min} 237 nm; (0.1 N NaOH), λ_{max} 278 nm (ε 19 500), λ_{min} 259 nm; (0.1 N HCl) λ_{max} 270 nm (ε 17 000), λ_{min} 239 nm; EIMS m/e 843 (M^+ − C_4H_9, 1), 613 (M^+ − SipR, 0.2), 287 (SipR, 6); CIMS (NH_3) m/e 901 (M^+ + 1, 0.06), 843 (0.13), 287 (SipR, 13); CIHRMS (NH_3) m/e 901.4368 ($C_{44}H_{69}N_4O_{12}Si_2$ requires 901.44503); EIHRMS m/e 843.3666 ($C_{40}H_{59}N_4O_{12}Si_2$ (M^+ − C_4H_9 requires 843.3664); EIHRMS m/e 613.2676 ($C_{30}H_{41}N_4O_8$Si (M^+ + 1 − ([$C_{14}H_{27}O_4$Si] = SipR)) requires 613.2691); EIHRMS m/e 287.1660 ($C_{14}H_{27}O_4$Si (M^+ + 1 − [$C_{30}H_{41}N_4O_8$Si] = SipR) requires 287.1677).

The Minor Dimer Product 31. This compound was a product of the pyrolysis reaction of **6**, R_f(A) = 0.49. It was isolated using HPLC (retention time above = 33.2 min): ^{1}H NMR (500 MHz; homonuclear decoupling; double quantum filter phase-sensitive COSY) δ 7.78 (bs, 1 H, NH_a), 7.03 (bs, 1 H, NH_b), 6.65 (m, 1 H, C=CHC*H*=CH_2), 5.53 and 5.50 (2 m, 2 H, C2′$_a$-H and C1′$_a$-H), 5.47 (d, J = 10.3 Hz, 1 H, CCHCH=C*H*$_2$), 5.40 (d, J = 17.4 Hz, 1 H, C=CHCH=C*H*$_2$), 5.38 (d, J = 9.7 Hz, 1 H, C=CHCH=C*H*$_2$), 5.13 (d, J = 17.6 Hz, 1 H, CCHCH=C*H*$_2$), 5.10 (s, 1 H, C1′$_b$-H), 5.08 (d, J = 6.4 Hz, 1 H, C2′$_b$-H), 4.87 (dd, J = 6.3, 2.9 Hz, C3′$_a$-H), 4.72 (dd, J = 6.2, 1.1 Hz, C3′$_b$-H), 4.11 (m, 1 H, C4′$_a$-H), 3.98 (m, 1 H, C4′$_b$-H), 3.80 and 3.71 and 3.70 (m, 5 H, C5′$_a$-H and C5′$_b$-H and CC*H*CH=CH_2), 3.16 (m, 1 H, CH_2C*H*$_2$), 2.59 (m, 1 H, CH_2C*H*$_2$), 2.08 (m, 1 H, C*H*$_2$CH$_2$), 1.85 (m, 1 H, C*H*$_2$CH$_2$), 1.51 (s, 3 H, C(C_aH_3)$_2$), 1.42 (s, 3 H, C(C_bH_3)$_2$), 1.37 (s, 3 H, C(C_aH_3)$_2$), 1.29 (s, 3 H, C(C_bH_3)$_2$), 0.88 (s, 9 H, C(C_aH_3)$_3$), 0.83 (s, 9 H, C(C_bH_3)$_3$), 0.10 (s, 3 H, (C_aH_3)$_2$Si), 0.06 (s, 3 H, (C_aH_3)$_2$Si), −0.02 (s, 3 H, C_bH_3Si), −0.03 (s, 3 H, C_bH_3Si); UV (MeOH) λ_{max} = 262 nm, λ_{min} = 237 nm; EIMS m/z 843 (2), 613 (M^+ − SipR, 1), 287 (SipR, 2); CIMS (NH_3) m/z 901 (M^+ + 1, 4), 843 (4), 287 (SipR, 96); EIHRMS m/e 843.3668 ($C_{40}H_{59}N_4O_{12}Si_2$ (M^+ − C_4H_9) requires 843.3664); EIHRMS m/e 613.2683 ($C_{30}H_{41}N_4O_8$Si (M^+ + 1 − ([$C_{14}H_{27}O_4$Si]=SipR)) requires 613.2691).

2′,3′-*O*-Isopropylidine-5-(hydroxymethyl)-6-allyluridine (33). Dimethyl acetal **28** (123 mg, 0.239 mM) was stirred in 5 mL of acetic acid/water (4/1) for 1 h. Solvent removal afforded 2′,3′-*O*-isopropylidine-5-formyl-6-allyluridine (**32**) as a residue that was not purified further, R_f(A) = 0.30.

Sodium borohydride (200 mg, 5.3 mM) was added to the residue (**32**) in 7 mL of THF/water (4/1) at 0 °C. The solution was stirred for 10 min at 0 °C and 20 min at rt. The temperature was lowered to 0 °C, and 2 g (37 mM) of ammonium chloride was added. The mixture was stirred for 10 min at 0 °C and 20 min at rt. After solvent removal, the residue was purified by radial chromatography (2 mm SiO_2, eluant: methylene chloride/MeOH gradient) to afford 78 mg (84 mg theor, 92% from **28**) of **33** as a foam, R_f(B) = 0.53.

2′,3′-*O*-Isopropylidine-5-methyl-6-allyluridine (35). Diol **33** (21 mg, 0.06 mM) and 12 mg (0.065 mM) of (thiocarbonyl)-diimidazole were stirred in 5 mL of THF overnight. Toluene (3 mL), tri-*n*-butyltin hydride (0.04 mL, 0.14 mM), and a catalytic amount of AIBN were added. After heating at reflux overnight, solvent was removed to afford a residue. Purification by thick-layer chromatography (1 mm SiO_2, ethanol/methylene chloride = 1/9) to afforded only **33** and 1.5 mg (20 mg theor, 8%) of **35** as a film: R_f(A) = 0.21; ^{1}H NMR (500 MHz) δ 8.25 (s, 1 H, NH), 5.88 (m, 1 H, C*H*=CH$_2$), 5.59 (d, J = 2.6 Hz, 1 H, C1′-H), 5.30 (d, J = 10.3 Hz, 1 H, CH=C*H*$_2$), 5.24 (dd, J = 6.4, 2.7 Hz, 1 H, C2′-H), 5.15 (d, J = 17.3 Hz, 1 H, CH=C*H*$_2$), 5.03 (dd, J = 6.6, 3.7 Hz, 1 H, C3′-H), 4.19 (dd, J = 6.2, 3.5 Hz, 1 H, C4′-H), 3.85 (dd, J = 12.2, 2.5 Hz, 1 H, C5′-H_a), 3.74 (dd, J = 12.2, 3.6 Hz, 1 H, C5′-H_a), 3.43 (dd, J = 17.3, 5.1 Hz, 1 H, C*H*$_2$CH=CH$_2$), 3.37 (dd, J = 17.3, 5.1 Hz, 1 H, C*H*$_2$CH=CH$_2$), 1.95 (s, 3 H, C5-CH_3), 1.52 (s, 3 H, CH_3), 1.32 (s, 3 H, CH_3); ^{13}C NMR (125 MHz) δ 162.62 (C-4), 150.64 (C-2), 148.50 (C-6), 129.88 (*C*H=CH$_2$), 119.07 (CH=*C*H$_2$), 114.09 (*C*(CH_3)$_2$), 93.04 (C-1′), 87.27 (C-4′), 83.06 (C-2′), 80.49 (C-3′), 62.95 (C-5′), 33.48 (CH$_2$CH=CH$_2$), 27.32 (CH_3), 25.27 (CH_3), 10.76 (C5-CH_3); UV (MeOH) λ_{max} = 267 nm, λ_{min} = 237 nm; CIHRMS (NH_3), m/e 339.1545 ($C_{16}H_{23}N_2O_6$ requires 339.1556).

5′-Deoxy-2′,3′-*O*-isopropylidine-5-methyl-6-allyluridine (37). Methyltriphenoxyphosphonium iodide (53.3 mg, 0.12 mM) in 0.2 mL of DMF at 0 °C was added to diol **33** (3.8 mg, 0.011 mM) in 0.2 mL of THF under argon. After 10 min, 0.2 mL of MeOH was added, and the solution stirred at 0 °C for 10 min. The solvent was removed under reduced pressure without heating, the flask was placed in ice, and 2 mL each of methylene chloride (0 °C) and saturated aqueous sodium thiosulfate (0 °C) were added. The aqueous layer was removed, the organic layer was washed with water (2 × 2 mL, 0 °C), and solvent was removed under vacuum without heating to afford a residue containing 2′,3′-*O*-isopropylidine-5′,5-bis(iodomethyl)-6-allyluridine (**36**): CIMS (NH_3) m/e 575 (M^+ + 1, 1).

Half of the residue **36** (ca. 5 μM) was suspended in 1 mL of benzene under argon, and tri-*n*-butyltin hydride (0.011 mL, 35 μM) and a catalytic amount of AIBN were added. The mixture was irradiated for 3 h. HPLC analysis and purification (retention time = 25.9 min) of the reaction mixture indicated **37** comprised 100% of the solution (70% when 21 μM of tri-*n*-butyltin hydride was used). **37**: R_f(C) = 0.58; ^{1}H NMR (500 MHz) δ 7.95 (s, 1 H, NH), 5.89 (m, 1 H, C*H*=CH$_2$), 5.58 (s, 1 H, C1′-H), 5.29 (d, *J* = 10.6 Hz, 1 H, CH=C*H*$_2$), 5.20 (d, *J* = 6.8 Hz, 1 H, C2′-H), 5.15 (d, *J* = 17.1 Hz, 1 H, CH=C*H*$_2$), 4.68 (t, *J* = 5.5 Hz, 1 H, C3′-H), 4.11 (m, 1 H, C4′-H), 3.44 (m, 1 H, C*H*$_2$CH=CH$_2$), 3.36 (dd, *J* = 17.3, 5.1 Hz, 1 H, C*H*$_2$CH=CH$_2$), 1.94 (s, 3 H, C5-CH$_3$), 1.50 (s, 3 H, CH$_3$), 1.35 (d, *J* = 6.3 Hz, 3 H, C5′-H) 1.30 (s, 3 H, CH$_3$); UV (MeOH) λ_{max} = 267 nm, λ_{min} = 244 nm; EIHRMS *m/e* 322.1531 ($C_{16}H_{22}N_2O_5$ requires 322.15273).

2′,3′-*O*-Isopropylidine-5′-*O*-((*tert*-butyldimethylsilyl)-oxy)-5-methyl-6-allyluridine (11). To **35** (<1 mg, <0.011 mM) dissolved in 1 mL of DMF under argon was added 0.05 mL (0.36 mM) of triethylamine, a catalytic amount of DMAP, and 20 mg (0.13 mM) of *tert*-butyldimethylsilyl chloride. After the mixture was stirred overnight, 1 mL of water was added and the solution stirred for 1 h. The solvents were removed, leaving a residue that was dissolved in 4 mL of hexane/water (1/1). After removal of the organic layer, the aqueous layer was washed with hexane (2 × 2 mL), and the organic layers were pooled. Removal of the solvent afforded a residue that was resuspended in chloroform and purified by HPLC (retention time = 10.0 min) to afford <1 mg (quantitative by TLC) of **11** as a film: ^{1}H NMR (500 MHz) δ 7.84 (s, 1 H, NH), 5.89 (m, 1 H, C*H*=CH$_2$), 5.65 (s, 1 H, C1′-H), 5.28 (d, *J* = 10.1 Hz, 1 H, CH=C*H*$_2$), 5.17 (dd, *J* = 5.7, 1.3 Hz, 1 H, C2′-H), 5.12 (d, *J* = 17.3 Hz, 1 H, CH=C*H*$_2$), 4.77 (dd, *J* = 5.3, 4.2 Hz, 1 H, C3′-H), 4.11 (dt, *J* = 6.2, 5.3 Hz, 1 H, C4′-H), 3.78 (dd, *J* = 8.0, 5.3 Hz, 1 H, C5′-H$_a$), 3.74 (dd, *J* = 9.0, 7.3 Hz, 1 H, C5′-H$_b$), 3.42 (m, 2 H, C*H*$_2$CH=CH$_2$), 1.94 (s, 3 H, C5-CH$_3$), 1.50 (s, 3 H, CH$_3$), 1.30 (s, 3 H, CH$_3$); UV (MeOH) λ_{max} = 268 nm, λ_{min} = 235 nm; CIHRMS (NH$_3$) *m/e* 453.239 ($C_{22}H_{37}N_2O_6Si$ requires 453.2421).

Acknowledgment. The authors are indebted to Debra Schneider, Rondall Allen, Todd Houston, and David Howard for technical assistance, to Dr. R. Singh and Professor R. Givens for assistance with the tin reactions, to Dr. D. Vander Velde for assistance with the 2-D NMR studies, to Professors T. Engler, G. Georg, and J. Aubé for helpful discussions, to Professor D. Rich and Drs. J. V. N. Vara Prasad and E. Veliz for assistance in preparation of the manuscript, and to Dr. C. Judson, R. Drake, and Dr. T. Williams for obtaining the mass spectral data. Funding provided by the NCI Training Grant and NIH CA 07522 is greatfully acknowledged.

Supplementary Material Available: Characterization data for **21**, **23–28**, **32**, and **33** as well as ^{1}H NMR spectra for compounds **6**, **11**, **23**, **27**, **29**, **30**, **31**, **33**, **35**, and **37** (16 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.