Synthesis and characterization of a ribavirin-3',5'-phosphate pentadecamer homoribopolymer bearing a 5'-amino tether group and a 3'-thymidine

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ABSTRACT

The synthesis of a pentadecamer of the 5'-phosphate of the antiviral nucleoside ribavirin (1'- β -D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide) has been achieved. This homoribopolymer is terminated at the 5'-position with an (6-aminohexyl)phosphate group to permit conjugation to a carrier and at the 3'-position by a thymidine-5'-phosphate. The synthesis was accomplished using the methyl phosphoramidite approach to oligoribonucleotides. The homoribopolymer was insensitive to ribonuclease A but was sensitive to ribonuclease T₂ digestion.

INTRODUCTION

Ribavirin (1'- β -D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide, **1**) is a synthetic nucleoside with a broad spectrum of antiviral activity (1, 2). Ribavirin exerts its antiviral effects by inhibiting viral replication and protein synthesis, a process requiring phosphorylation at the 5'-position of the sugar ring to produce mono-, di-, and triphosphates (3, 4). We report here the synthesis of a polymeric form of ribavirin-5-phosphate, 6-aminohexyl-p-(Rp)₁₅(3'-5')T (**10**). Such an oligomer could be useful in targeted antiviral drug therapy using monoclonal antibodies by permitting a higher loading of drug on its carrier. Selective targeting to virally infected cells would enhance therapeutic efficacy by reducing systemic toxicity or by increasing drug concentration at the target cell (5, 6). After internalization of the immunodrug conjugate by endocytosis, release of ribavirin or its 5'-phosphate could occur by cleavage of the oligomer by lysosomal phosphatases or nucleases. The 6-aminohexylphosphate group was employed as the tether group at the 5'-terminus of the oligomer to permit conjugation to an antibody. A thymidine was introduced at the 3'-terminus to provide a chromophore to facilitate quantitation and purification because of the relatively low UV absorbance of the triazole ring of ribavirin [$\epsilon_{206\text{ nm}} = 1.2 \times 10^4$ (water)]. The synthesis of this oligomer required the preparation of an appropriately protected ribavirin phosphoramidite **6** from **1**, which would be amenable to automated solid-phase oligoribonucleotide synthesis.

RESULTS AND DISCUSSION

The 2'-hydroxyl group of the ribose ring of **1** required protection during the synthesis of the oligoribonucleotide. Both tetrahydropyranyl (THP) (7) and *t*-butyldimethylsilyl (TBDMS) (8, 9) protecting groups have been used for this purpose. The 3'- and 5'-hydroxyl groups of ribavirin were first blocked as the cyclic 1,1,3,3-(*iso*-propyl)₄disiloxanyl ether to permit introduction of the THP group at the 2'-hydroxyl. Unfortunately, the amine group of the amide also reacted to give the bis-THP ether. Selective removal of the amide THP was not successful although adducts of alkylamines with dihydropyran are reported to be very acid labile (9). Therefore, the protecting group strategy employed by Usman *et al.* (8) was adapted. The 5'-hydroxyl of **1** was selectively protected as the 4,4'-dimethoxytrityl ether (Figure 1). Steric hindrance by the heterocyclic rings of the natural nucleosides permits highly selective silylation with TBDMS chloride at the 2'-hydroxyl position (10, 11). The triazolecarboxamide ring of **1** did not provide this selectivity with either of the two silylation procedures investigated. TBDMS chloride-imidazole in DMF (10) afforded a mixture of the 2'-*O*-TBDMS (**3**), 3'-*O*-TBDMS (**4**), and 2',3'-*O*-TBDMS (**5**) protected ethers in a 3:13:27 ratio, whereas TBDMS chloride-AgNO₃ in pyridine (11) was more selective and afforded a 32:8:9 mixture of these ethers. The position of silylation was determined by ¹H NMR spectroscopy. Because silylation deshields the adjacent proton (12), the position of 2'-H in the 2'-*O*-silylated derivative **3** was shifted downfield to 4.57 from 4.40 ppm of that of **1** and that for the 3'-H in the 3'-*O*-silylated derivative **4** was shifted to 4.38 from 4.32 ppm in **1**. The 3'-*O*-mono- and 2',3'-*O*-disilyl ethers were readily cleaved with (*n*-Bu)₄NF to regenerate **2**, which was recycled.

With the 2'- and 5'-hydroxyl groups protected, the 3'-hydroxyl was converted to the phosphoramidite (Figure 1), by reaction with *N,N*-diisopropylmethylphosphoramidite chloride (1.3 equiv.) in the presence of (*i*-Pr)₂NEt (4.0 equiv.) and DMAP (0.2 equiv.). Chromatography afforded a 71% yield of 5'-*O*-(4,4'-dimethoxytrityl)-2'-*O*-(*t*-butyldimethylsilyl)-1'- β -D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide 3'-(*N,N*-di-*iso*-propyl)methylphosphoramidite (**6**) as a diastomeric mixture (1:0.6) as evidenced by ¹H and ³¹P NMR spectroscopy. The ³¹P NMR spectrum showed two signals for phosphorus at 147.2 and 148.6 ppm.

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Figure 1. Preparation of phosphoramidite **6**. Reagents: (a) DMTTrCl, C_5H_5N ; (b) TBDMSCl, $AgNO_3$, C_5H_5N ; (c) $(n-Bu)_4NF$, THF; (d) **3**, $(i-Pr)_2NP(OMe)Cl$, $(i-Pr)_2NEt$, DMAP, THF.

Figure 2. Preparation of the ribavirin phosphate pentadecamer 6-aminoethyl-p-(Rp)₁₅(3'-5')T (triethylammonium salt) (**10**) bearing an amino-functionalized 5'-tether group and a 3'-(thymidine-5'-phosphate) ($R = \text{ribavirin}$). Reagents: (a) TCA, CH_2Cl_2 ; (b) **6**, 1*H*-tetrazole, MeCN; (c) Ac_2O , DMAP, 2,6-lutidine, THF; (d) I_2 , C_5H_5N , THF, H_2O ; (e) steps a to d repeated 14 times; (f) TCA, CH_2Cl_2 ; (g) [MMTNH(CH₂)₆O]P[N(*i*-Pr)₂](OCH₂CH₂CN), MeCN; (h) Ac_2O , DMAP, 2,6-lutidine, THF; (i) I_2 , C_5H_5N , THF, H_2O ; (j) C_6H_5SH , Et_3N , dioxane; (k) 95% EtOH; (l) NH_4OH , 95% EtOH; (m) $(n-Bu)_4NF$, THF; (n) 80% HOAc.

The ribavirin phosphate homopolymer (**10**, Figure 2) was prepared on an automated DNA synthesizer using a 1- μ mole DNA synthesis cycle, with extended coupling-wait times to accommodate the decreased reactivity of the hindered phosphoramidite **6**, in order to add 15 ribavirin-3'-phosphate residues to the initial thymidine residue coupled to the controlled pore glass support. [6-(*N*-4-Methoxytritylamino)hexyl](*N,N*-diisopropyl)-2-cyanoethylphosphoramidite was used to introduce the terminal tether group. The trityl group on the tether was retained to facilitate purification of the oligomer. The methyl

protecting groups on the ribavirin phosphates were removed using $C_6H_5SH-Et_3N$, and the 2-cyanoethyl group on the terminal 5'-phosphate linking the tether to the oligomer chain was cleaved during the NH_4OH treatment used for removing the oligomer from the support. Treatment with $(n-Bu)_4NF$ removed the TBDMS groups. After polyacrylamide gel electrophoresis (PAGE) to remove truncated sequences and gel filtration (Sephadex G-15), HOAc was used to cleave the trityl group from the 5'-terminus of the oligomer. Reverse-phase chromatography and subsequent lyophilization afforded **10** as a white solid in 13% overall yield, based on the absorption of thymidine at 260 nm. HPLC on a MA7Q anion-exchange column showed a single peak (>98%). 1H NMR spectroscopy indicated that the fluoride treatment removed 98% of the 2'-O-TBDMS protecting groups. A multiplet at 1.2–1.7 ppm was ascribed to the four central methylene groups of the tether. A singlet for the methyl group on the thymidine pyrimidine ring integrating for 3 protons occurred at 1.89 ppm. The anomeric proton of the ribose ring of ribavirin produced a doublet at 5.95 ppm that integrated for 15 protons. The corresponding proton of the furanose ring of thymidine produced a triplet at 6.27 ppm that integrated for one proton. The singlet at 8.69 ppm for the 5-H of the triazole moiety of ribavirin integrated for 15 protons. Comparison of the integral for this proton with that for the 4'-H of ribose indicated that depurination was negligible (<1%). The relative integrals for the thymidine and ribavirin heterocyclic ring protons verified the presence of 15 ribavirin units per thymidine.

The stability of **10** to alkaline and enzymatic hydrolysis was determined. Incubation of **10** with 0.5 N KOH at 37°C for 1 h resulted in approximately 20% hydrolysis as determined by HPLC (MA7Q anion-exchange). Incubation for an additional 5 h at 37°C led to disappearance of the UV-absorbing peak corresponding to **10**. Homoribopolymer **10** was resistant to cleavage by ribonuclease A (1.3 mM Tris-HCl, pH 7.5, 1.3 mM $MgCl_2$, 37°C, 15 h) but was cleaved completely by ribonuclease T₂ (0.04 mM NaOAc, 37°C, 30 min).

MATERIALS AND METHODS

Reagents and Instrumentation

Anhydrous reactions were conducted under argon. Anhydrous solvents were distilled under argon before use. Melting points were uncorrected. TLC analyses were performed using Analtech analytical silica gel plates. Merck silica gel 60 was used for preparative chromatography. IR spectra were recorded using a Perkin-Elmer 710B infrared spectrometer, and NMR spectra using JEOL FX90Q and 400-MHz Varian spectrometers. UV spectra were recorded on a Perkin-Elmer 575 spectrometer. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, TN.

5'-O-(4,4'-Dimethoxytrityl)-1- β -D-ribofuranosyl-1*H*-1,2,4-triazole-3-carboxamide (**2**). To a stirred suspension of 3.00 g (12.3 mmol) of **1** (**2**) in 40 mL of anhydrous C_5H_5N (distilled from CaH_2) was added, in four portions at 1-h intervals, 4.83 g (14.1 mmol) of 4,4'-dimethoxytrityl chloride (DMTrCl). The dark-orange reaction mixture was stirred for 18 h at room temperature under Ar and then concentrated. The residue was diluted with CH_2Cl_2 (300 mL), washed with 5% aq. $NaHCO_3$ (200 mL), which was then back-extracted with CH_2Cl_2 (100 mL), washed with water (100 mL), and dried (Na_2SO_4). Concentration

afforded 10.7 g of a dark-yellow gum, which was chromatographed (200 g of silica gel, 4.5×46 cm column, 2.5 L of 0–10% MeOH/CH₂Cl₂ gradient) to give in fractions 11 to 14 (500-mL) 5.09 g (76%) of **2** as a white solid: TLC (5% MeOH/CH₂Cl₂) R_f 0.16; IR (KBr) 3000–3500, 1700, 1620, 1520, 1180, 1030, 830 cm⁻¹; ¹H NMR (Me₂SO-d₆) δ 3.10 (m, 2H, 5'-CH₂), 3.71 (s, 6H, OCH₃), 4.03 (m, 1H, 4'-CH), 4.32 (dd, J = 7 Hz, 1H, 3'-CH), 4.40 (m, 1H, 2'-CH), 5.19 (d, J = 6 Hz, 1H, OH), 5.64 (d, J = 5 Hz, 1H, OH), 5.92 (d, J = 3 Hz, 1H, 1'-CH), 6.8 (m, 4H, ArH), 7.2 (m, 7H, ArH), 7.3 (m, 2H, ArH), 7.62 and 7.75 (2s, 2H, NH₂), 8.84 (s, 1H, 5-ArH). Anal. calcd. for C₂₉H₃₀N₄O₇: C, 63.74; H, 5.49; N, 10.26. Found: C, 63.69; H, 5.52; N, 10.17.

5'-O-(4,4'-Dimethoxytrityl)-2'-O-(t-butyltrimethylsilyl)-1'-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide (3). To a solution of 10.25 g (18.8 mmol) of **2** in 20 mL of THF (distilled from K-C₆H₅COC₆H₅) and 7.0 mL (87 mmol) of anhydrous C₅H₅N was added 3.81 g (22.4 mmol) of AgNO₃ (11). The reaction mixture was stirred for 30 min at ice-bath temperature before 3.66 g (24.3 mmol) of TBDMSCl was added. A white emulsion formed instantly. The reaction mixture was slowly warmed to room temperature and stirred for 2 h under Ar, while protected from light, and filtered into 5% aq. NaHCO₃ (75 mL). The filtrate was extracted with CH₂Cl₂ (3×75 mL). The extracts were washed with H₂O (3×50 mL), dried (Na₂SO₄) overnight, and concentrated to give 15 g of a white foam, which was chromatographed (180 g of silica gel, 4.5×38 cm column, 4 L of 0–100% EtOAc/CH₂Cl₂ gradient) twice to give in the following order: 1.38 g (9%) of the disilylated derivative **5**, 3.99 g (32%) of the desired 2'-O-monosilylated **3**, and 0.75 g (8%) of the 3'-O-monosilylated ether **4**.

5'-O-(4,4'-Dimethoxytrityl)-2'-O-(t-butyltrimethylsilyl)-1'-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide (3): TLC R_f 0.52; IR (KBr) 3000–3500, 2980, 2880, 1680, 1600, 1520, 1460, 1260, 1180, 1040 cm⁻¹; ¹H NMR (Me₂SO-d₆) δ 0.02 (2s, 6H, SiCH₃), 0.81 (s, 9H, SiC(CH₃)₃), 3.00 (m, 2H, 5'-CH₂), 3.71 (s, 6H, OCH₃), 4.05 (m, 1H, 4'-CH), 4.28 (m, 1H, 3'-CH), 4.57 (m, 1H, 2'-CH), 5.16 (m, 1H, 3'-OH, exchangeable with D₂O), 5.93 (m, 1H, 1'-CH), 6.85 (m, 4H, ArH), 7.22 (m, 7H, ArH), 7.35 (m, 2H, ArH), 7.67 and 7.68 (2s, 2H, NH₂), 8.89 (s, 1H, 5-ArH). Anal. calcd. for C₃₅H₄₄N₄O₇Si: C, 63.64; H, 6.67; N, 8.48. Found: C, 63.31; H, 7.05; N, 8.24.

5'-O-(4,4'-Dimethoxytrityl)-3'-O-(t-butyltrimethylsilyl)-1'-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide (4): TLC R_f 0.16; IR (KBr) 3000–3500, 2980, 2880, 1700, 1620, 1580, 1520, 1460, 1260, 1180, 1040, 840 cm⁻¹; ¹H NMR (Me₂SO-d₆): δ 0.02 and 0.04 (2s, 6H, SiCH₃), 0.78 (s, 9H, SiC(CH₃)₃), 3.00 (m, 2H, 5'-CH₂), 3.70 (s, 6H, OCH₃), 4.00 (m, 1H, 4'-CH), 4.38 (m, 1H, 3'-CH), 4.50 (m, 1H, 2'-CH), 5.50 (m, 1H, 2'-OH, exchangeable with D₂O), 5.90 (m, 1H, 1'-CH), 6.85 (m, 4H, ArH), 7.20 (m, 7H, ArH), 7.32 (m, 2H, ArH), 7.63 and 7.64 (2s, 2H, NH₂), 8.90 (s, 1, 5-ArH). Anal. calcd. for C₃₅H₄₄N₄O₇Si: C, 63.64; H, 6.67; N, 8.48. Found: C, 63.35; H, 6.83; N, 8.17.

5'-O-(4,4'-Dimethoxytrityl)-2',3'-di-O-(t-butyltrimethylsilyl)-1'-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide (5): TLC R_f 0.77; IR (KBr) 3000–3500, 2960, 2880, 1700, 1640, 1520,

1460, 1270, 1190, 1050, 840 cm⁻¹; ¹H NMR (Me₂SO-d₆) δ 0.01, 0.06, 0.09, and 0.20 (4s, 12H, SiCH₃), 0.75 and 0.78 (2s, 18H, SiC(CH₃)₃), 3.05 (dd, J = 4 Hz, 2H, 5'-CH₂), 3.71 (s, 6H, OCH₃), 4.00 (m, 1H, 4'-CH), 4.20 (m, 1H, 3'-CH), 4.65 (m, 1H, 2'-CH), 5.90 (d, J = 5 Hz, 1H, 1'-CH), 6.88 (m, 4H, ArH), 7.28, (m, 7H, ArH), 7.38 (m, 2H, ArH), 7.60 and 7.68 (2s, 2H, NH₂), 8.96 (s, 1H, 5-ArH). Anal. calcd. for C₄₁H₅₈N₄O₇Si₂: C, 63.56; H, 7.49; N, 7.24. Found: C, 63.79; H, 7.34; N, 7.66.

5'-O-(4,4'-Dimethoxytrityl)-2'-O-(t-butyltrimethylsilyl)-1'-β-D-ribofuranosyl-1H-1,2,4-triazole-3-carboxamide 3'-(N,N-di-isopropyl)methylphosphoramidite (6). A published procedure (10) was adapted. A solution of 3.7 g (5.6 mmol) of **3** in 25 mL of dry (tetrahydrofuran) THF was added dropwise under Ar over a period of 2 h to a solution of 3.9 mL (2.9 g, 23 mmol) of (*i*-Pr)₂NEt, 1.40 mL (7.2 mmol) of (*i*-Pr)₂NP(OMe)Cl and 140 mg (1.14 mmol) of DMAP in 20 mL of THF (distilled from Na-C₆H₅COC₆H₅). The cloudy solution was stirred at room temperature under Ar for 20 h. The reaction mixture containing precipitated salts was diluted with EtOAc (250 mL) and washed with sat. NaCl (5×50 mL). The slightly yellowish organic extract was dried (Na₂SO₄) and concentrated to give 4.64 g of a pale-yellow foam. This material was chromatographed (3×20 cm silica gel, 600 mL of 55% CH₂Cl₂:35% C₆H₁₄:10% Et₃N) to give after evaporation 3.27 g (71%) of **6** as a colorless foam: TLC (2% Et₃N:49% EtOAc:49% C₆H₁₄) R_f 0.44; IR (KBr) 3450, 2650–2950, 1690, 1520, 1480, 1260, 1200, 1040, 840 cm⁻¹; ¹H NMR (CDCl₃) [diastereomeric pairs of signals (1:0.6)] δ -0.08, -0.06, 0.04, and 0.06 (4s, 6H, Si(CH₃)₂), 0.82, 0.83, 0.98, and 1.00 (4s, 9H, SiC(CH₃)₃), 1.15 and 1.28 (2m, 12H, N[CH(CH₃)₂]₂), 3.12 and 3.14 (2m, 2H, 5'-CH₂), 3.55 (m, 2H, N[CH(CH₃)₂]₂), 3.79 (3s, 9H, ArOCH₃, POCH₃), 4.28 and 4.30 (2m, 1H, 4'-H), 4.34 and 4.40 (2m, 1H, 3'-H), 4.92 and 4.98 (2m, 1H, 2'-H), 5.82 and 5.88 (2d, J = 6 Hz, 1H, 1'-H), 6.80 (m, 4H, ArH), 7.24 (m, 7H, ArH), 7.40 (m, 2H, ArH), 7.52 (2d, J = 1.5 Hz, 2H, NH₂), 8.38 and 8.40 (2s, 1H, 5-ArH); ³¹P NMR (CDCl₃) (internal standard 85% H₃PO₄) 147.21 and 148.61 ppm. Anal. calcd. for C₄₂H₆₀N₅O₈PSi: C, 61.38; H, 7.31; N, 8.53. Found: C, 61.31; H, 7.58; N, 8.38.

6-Aminohexyl-p-(Rp)₁₅(3'-5')T: 5'-(6-Aminohexyl)-3'-thymidylate-[1-(5-O-phosphono-β-D-ribofuranosyl)-1H-1,2,4-triazole-3-carboxamide]pentadecamer (N,N-diethylethanamine salt) (10)*. A published procedure (10) was adapted. The oligoribonucleotide was synthesized using standard solid-phase 2-cyanoethyl phosphoramidite chemistry on an Applied Biosystems Model 380A DNA Synthesizer. The 1-μmole DNA synthesis cycle was modified with a coupling-wait time of 8 min, using 0.1M ribavirin phosphoramidite **6** in MeCN. Twenty-seven 1-μmole thymidine-CPG columns were employed for the synthesis using 15 cycles with **6**, followed by one with [6-(N-4-methoxytritylamino)-hexyl](N,N-di-iso-propyl)-2-cyanoethylphosphoramidite. Release of the DMTr cation was monitored by measuring absorbance at 498 nm of the trityl fractions diluted with 0.1M TsOH in MeCN. The coupling efficiency averaged 94%. The terminal 4-MeOTr group was retained to aid in visualization during -PAGE purification. The methyl protecting groups of the phosphate functions were removed by standard C₆H₅SH-Et₃N treatment. After cleavage of the oligomer from the column with conc. NH₄OH-95% EtOH and lyophilization, the residue was treated with 7.0 mL of 1 M (*n*-Bu)₄NF in THF overnight to remove

the 2'-*O*-silyl protecting groups. The reaction mixture was diluted with 7.0 mL of 0.05 M Et₃NHOAc (TEA) and chromatographed on a 2.5×40-cm Sephadex G-15 column (Pharmacia) with 0.05 M TEA. The fully deprotected oligomer was eluted in the void volume as detected by absorption at 260 nm and then lyophilized. The residue was dissolved in 0.64 mL of 90% deionized HCONH₂ and purified by PAGE on nine preparative, 1.5-mm 20% acrylamide gels containing 7 M urea. The oligomer was eluted from the gels with 0.05 M TEA, rechromatographed on Sephadex G-15 with 0.05 M TEA, and lyophilized. The MMTr group was removed by treatment of the residue with 10 mL of 80% HOAc (v/v) for 3 h. After dilution with 50 mL of H₂O, the mixture was washed with Et₂O (3×25 mL) to remove nonpolar organic compounds and lyophilized. The yellow residue obtained was diluted with 12 mL of 0.05 M TEA and purified using 27 Sep-Pak cartridges (Waters Assoc.), pretreated with 10-mL volumes of MeOH and H₂O, by washing with H₂O followed by elution with 50% 0.05 M TEA in MeOH. Concentration and lyophilization to a constant weight afforded 24.7 mg of the TEA salt of **10** as a white solid, the absorbance at 260 nm of a solution of which in 50 mL of H₂O was 0.647. Based on the extinction coefficient of thymidine at 260 nm ($\epsilon = 8.8 \times 10^3$), the overall yield was 25.0 mg (13.6%): HPLC (50×7.8-mm Bio-Rad MA7Q anion-exchange column, gradient elution with 20 mM Tris-HCl, pH 7.5, to 20 mM Tris-HCl, pH 7.5, 500 mM NaCl, over 10 min at 1.0 mL/min, 260 nm) gave one peak (t_R 9.0 min); ¹H NMR (D₂O) δ 1.25 (t, J = 7.5 Hz, 144H, NCH₂CH₃), 1.2–1.7 (m, 8H, (CH₂)₄), 1.89 (s, 3H, thymidine CH₃), 2.02 (s, 3H, CH₃CO₂), 2.32 (t, J = 6 Hz, 2H, 2'-thymidine H), 2.92 (t, J = 8 Hz, 2H, NCH₂), 3.17 (q, J = 7.5 Hz, 96H, NCH₂CH₃), 3.34 (t, J = 8 Hz, 2H, CH₂OP), 4.09 (m, 32H, 5'-H), 4.22 (m, 1H, 4'-thymidine H), 4.33 (broad s, 15H, 4'-ribavirin H), 4.51 (m, 1H, 3'-thymidine H), 4.61 (t, J = 4 Hz, 15H, 3'-ribavirin H), 4.81 (m, 15H, 2'-ribavirin H), 5.95 (d, J = 4 Hz, 15H, 1'-ribavirin H), 6.27 (t, J = 6 Hz, 1H, 1'-thymidine H), 7.63 (s, 1H, thymidine 6-ArH), 8.69 (s, 15H, ribavirin 5-ArH). Evidence of <2% uncleaved TBDMS ether was established by the presence of a singlet at 0.92 ppm.

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