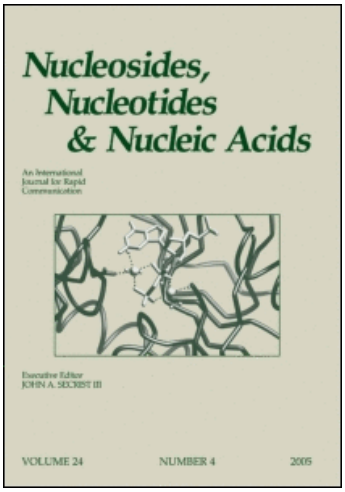


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# Synthesis of 5'-*C*-Branched Thymidines and Conversion to Phosphoramidites

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# SYNTHESIS OF 5'-*C*-BRANCHED THYMIDINES AND CONVERSION TO PHOSPHORAMIDITES

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**Abstract**: Thymidine was converted to its 5'-epoxy derivative, which was reacted with nucleophiles to give 5'-*C*-aminomethyl-, 5'-*C*-bromomethyl-, 5'-*C*-cyanomethyl, and 5'-*C*-methoxymethylthymidine derivatives with defined stereochemistry. 5'-*C*-ally-, 5'-*C*-hydroxymethyl-, 5'-*C*-hydroxypropyl-, and 5'-*C*-(imidazole-4-acetamido)methyl-thymidine derivatives were also prepared. The 5'-*C*-branched thymidines were converted to the corresponding phosphoramidites.



## Introduction

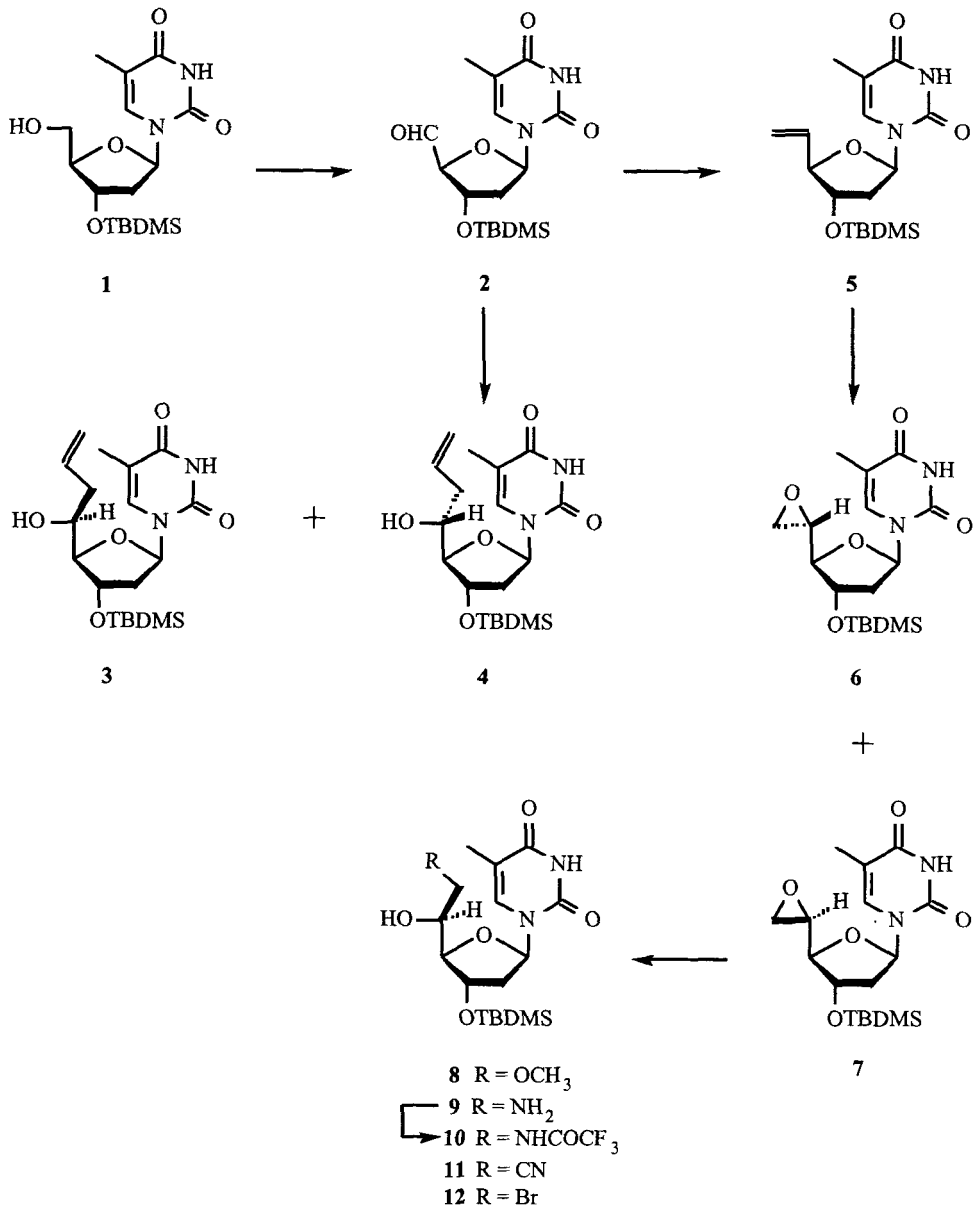
Antisense oligonucleotides as potential human therapeutics have been explored for a decade.[1-3] To have therapeutic importance, antisense oligonucleotides must be stable to a variety of cellular nucleases, hybridize efficiently and specifically to target RNA, and meet other requirements for human therapeutics. In efforts to meet these criteria, a variety of oligonucleotide analogs including backbone, base, and sugar modifications have been explored.[1,4-6] The early sugar modifications were focused on 2'-substitution of nucleosides and later, replacement of ribose by other sugar moieties drew considerable attention.[6] Among 2'-substitutions, 2'-*O*-alkyl- and 2'-fluorooligonucleotides exhibited excellent binding affinity to complementary RNA and improved stability to cellular nucleases.[7-9] Recently, oligonucleotides having *C*-branched sugar modifications have been synthesized and evaluated. Oligonucleotides containing 4'-*C*-substituted thymidines,[10-12] were reported to have satisfactory binding affinity and enzyme stability. Oligonucleotides containing 5'-*C*-branched nucleosides, reported by us and others in recent communication letters,[13-14] have also significantly increased enzyme stability while they retain sufficient hybridization to both DNA and RNA. Additionally, 5'-*C*-branched modifications provide potential conjugation sites for certain functional moieties such as alkylating agents, artificial nucleases, reporter molecules, etc. It seems that

oligonucleotides containing 5'-*C*-branched nucleosides are potentially useful in therapeutics and diagnostics and deserve further evaluation. With the extension of our sugar modification program, we have further explored 5'-*C*-branched sugar modifications in search of oligonucleotide analogs possessing sufficient stability to cellular nucleases and good binding affinity to RNA as well as the capability of activating RNase H. In this article, we will describe synthesis and stereochemistry of a number of 5'-*C*-branched thymidines and conversion to the corresponding phosphoramidites as oligonucleotide building blocks.

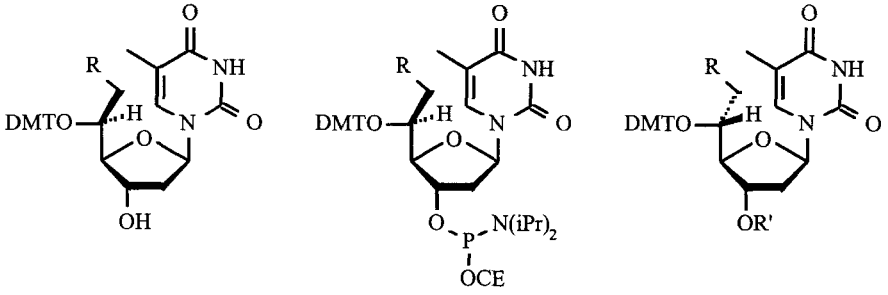
## Chemistry

5'-*C*-Branched thymidine derivatives were prepared from two key intermediates, 5'-formyl thymidine derivative **2** and 5'(S)-epoxy thymidine derivative **7** as shown in Scheme 1 and 2. **2** was prepared form **1** according to a published procedure.[15-16] **1** was prepared from thymidine in three steps[15] (a. DMT-Cl, pyridine; b. TBDMS-Cl, imidazole, pyridine; c. 80% AcOH) in 60% overall yield. Alternatively, **1** was also prepared from selective hydrolysis of 3',5'-*O*-bis(t-butyldimethylsilyl)thymidine with 80% AcOH in 55-65% yields. Reaction of **2** with allyl(triisopropoxy)titanium or allylmagnesium chloride in the presence of cuprous cyanide yielded a mixture of **3** and **4** in moderate to good yields. Wittig reaction of **2** with methyltriphenyl phosphorane gave the olefinic product **5**, which was oxidized with MCPBA to the epoxides **6** and **7** (ratio 1:9). The dominant formation of **7** could be ascribed to the selective attack of MCPBA from the less hindered side of the olefin, that is, the opposite side of the TBDMS. Nucleophilic additions to **7** by neutral or alkaline nucleophiles afforded 5'(*S*)-*C*-substituted thymidine derivatives with a defined configuration. Thus, a solution of **7** in methanol was refluxed over alumina to give **8** in 76% yield. Methanolysis of **7** in the presence of potassium carbonate afforded **8** in low yield owing to the instability of TBDMS group at the basic condition. **7** was treated with saturated ammonia in methanol to yield **9** exclusively, which was converted to **10** by treatment with S-ethyl thiotrifluoroacetate. Treatment of **7** with potassium cyanide at room temperature yielded **11** in good yield. However, the reaction at elevated temperature led to formation of a significant amount of by-products resulting from attack of cyanide on the C5' of the epoxide. Reaction of **7** with methylmagnesium bromide[17] yielded **12**, instead of 5'-*C*-ethylthymidine derivative, in good yield.

Reactions of **3**, **4**, **8**, **10**, and **11** with 4,4'-dimethoxytrityl chloride in the presence of silver triflate, followed by removal of TBDMS by TBAF, yielded **15**, **27**, **13**, **14**, and **18**, respectively, as shown in Scheme 2. **12** was subject to tritylation, replacement of bromo by acetoxy, and removal of TBDMS to give **16** in good yield. Hydrolysis of **14** with




Scheme 1



**13** R = $OCH_3$
**14** R = $NHCOCF_3$
**15** R = $CH{=}CH_2$
**16** R = OAc
**17** R = $NHCOCH_2$-4-DNP-imidazole
**18** R = CN

**19** R = $OCH_3$
**20** R = $NHCOCF_3$
**21** R = $CH{=}CH_2$
**22** R = OAc
**23** R = $NHCOCH_2$-4-DNP-imidazole
**24** R = CN

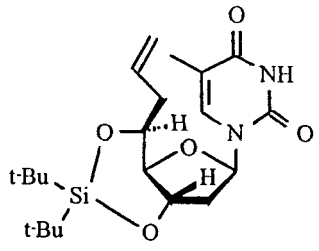
**25** R = $CH{=}CH_2$, R' = TBDMS
**26** R = $(CH_2)_2OH$, R' = TBDMS
**27** R = $CH{=}CH_2$, R' = H
**28** R = $(CH_2)_2OAc$, R' = H
**29** R = $CH{=}CH_2$, R' = $P(OCE)NiPr_2$
**30** R = $(CH_2)_2OAc$, R' = $P(OCE)NiPr_2$

Scheme 2



ammonium hydroxide yielded an amino derivative, which was treated with N-(2,4-dinitrophenyl)imidazole-4-acetic acid succinimide ester[18] to give **17**. Tritylation of **4** gave **25** and the subsequent hydroboration yielded **26**, which was subject to acetylation and removal of TBDMS to give **28**. Reactions of **13-18** and **27-28** with 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite yielded the phosphoramidites **19-24** and **29-30**, respectively.

We have previously reported the stereochemical assignment of the 5'-*C*-branched thymidines.[14] Recently, we have obtained additional NOE data that further confirm the previous assignment. Instead of 3',5'-*O*-TIPDS derivatives, 3',5'-*O*-di(t-butyl)silyl (DTBS) derivatives of 5'-*C*-allythymidines (**31** and **32**) were synthesized by reactions of 5'-*C*-allylthymidines with di(t-butyl)silyl ditriflate and used for NOE experiments. The chemical shifts of H3', H4', and H5' of **31** and **32** were assigned by COSY experiment. H3' and H5' were irradiated respectively and part of the reference spectra plotted as shown in Figure 1 and 2. Saturation of H3' of **32** resulted in significant NOE on H5' (Fig. 2. a) and saturation of H5' resulted in NOE on H3' (Fig. 2. b). However, saturation of H3' and H5' of **31** produced no NOE on H5' and H3'. The results are consistent with the previously reported,[14] indicating both DTBS and TIPDS[19] derivatives are useful for the assignment of stereochemistry of the 5'-*C*-branched nucleosides.




31

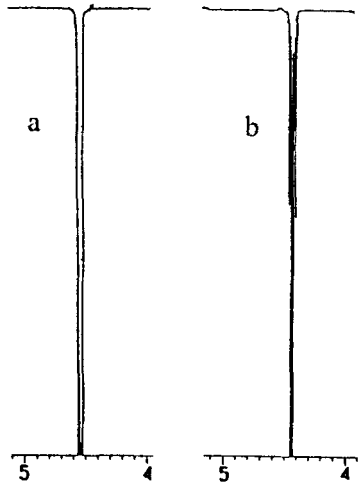
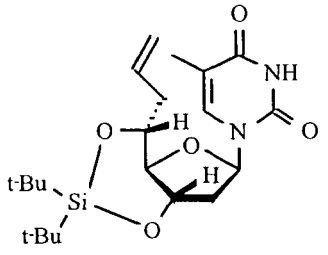



Figure 1. NOE of **31**: a) saturation of H3'; b) saturation of H5'.

Synthesis of oligodeoxynucleotides containing some of 5'-*C*-branched thymidines were described in our previous communication letter.[14] Similarly, the other 5'-*C*-branched thymidines were also incorporated into oligonucleotides. Detailed oligonucleotide synthesis and evaluation will be published elsewhere.

## Conclusion

Synthetic chemistry and stereochemistry of 5'-*C*-branched thymidines have been explored and are expected to apply to other nucleosides as well. A number of 5'(*S*)-*C*- and 5'(*R*)-*C*-branched thymidines were prepared and converted to the phosphoramidites as




**32**

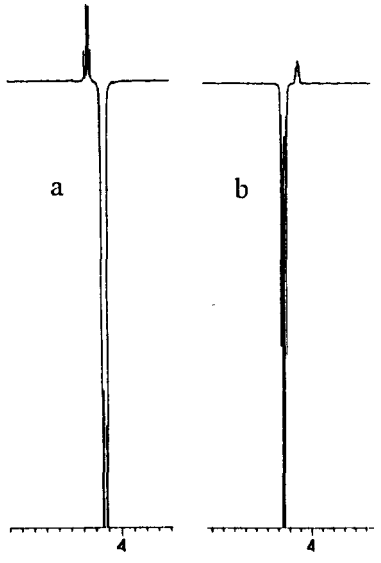



Figure 2. NOE of **32**: a) saturation of H3'; b) saturation of H5'.

oligonucleotide building blocks, which are potentially useful in oligonucleotide therapeutics and diagnostics.

## Experimental

NMR spectra ($^1$H and $^{31}$P) were recorded on a 500 MHz spectrometer and chemical shifts are reported in δ values (parts per million) with tetramethylsilane (TMS) as the internal standard for $^1$H and with phosphoric acid as the external standard for $^{31}$P. FAB mass spectra were obtained on a Fisons Autospec spectrometer. Elemental analysis was

conducted at NuMega Resonance Labs, San Diego. Melting points were measured with a capillary melting points apparatus and are uncorrected. Anhydrous solvents containing <0.005% water were purchased from Fluka or Aldrich and used directly without further treatment. Thin layer chromatography plates and silica gel for column chromatography were supplied by ICN.

**3'-*O*-(t-Butyldimethylsilyl)thymidine-5'-aldehyde 2.** A reaction mixture of thymidine (24.2 g, 0.1 mol), imidazole (20.4 g, 0.3 mol), and t-butyldimethylchlorosilane (37.7 g, 0.25 mol) in anhydrous pyridine (400 mL) was stirred at room temperature overnight. Pyridine was evaporated and the residue was diluted with ethyl acetate, washed with brine three times, dried ($Na_2SO_4$), and concentrated. The residue was dissolved in 80% AcOH (600 mL) and the resulting solution stood at room temperature for 5 days. Solvents were evaporated and the residue was passed through a short silica column with 30% ethyl acetate in hexanes as eluent to give 23.3 g (65.4%) of **1** as a white solid.



To a stirred solution of **1** (19.0 g, 0.053 mol), DCC (33.0 g, 0.16 mol), and pyridine (anhydrous, 4.3 mL, 0.053 mol) in anhydrous DMSO (190 mL) at 10 °C under nitrogen was added dropwise TFA (2.05 mL, 0.0267 mol) in DMSO (10 mL). The resulting reaction mixture was stirred at room temperature overnight and cooled to 10 °C. Water (40 mL) was added dropwise and the resulting mixture stirred at room temperature for 2 h. Precipitates were filtered and washed with DMSO. The combined filtrate was diluted with ethyl acetate, washed with brine five times, dried ($Na_2SO_4$), and concentrated. The crude was purified by chromatography on silica (EtOAc-hexanes 1:1) to give 15.9 g of **2** as a white solid; m.p. 91-93° (recrystallized from ethyl acetate/hexanes); $^1H$ NMR ($CDCl_3$) δ 9.75 (CHO), the other region of the spectrum was complicated by hydration; FABMS m/z 355 ($MH^+$); Anal. Calcd. for $C_{16}H_{26}N_2O_5Si \cdot {}^1/_2H_2O$: C, 52.86; H, 7.49; N, 7.71. Found: C, 53.00; H, 7.71, N, 7.41.

**3'-*O*-(t-Butyldimethylsilyl)-5'(*S*,*R*)-*C*-allylthymidines 3 and 4.**

Method A. To a suspension of anhydrous cuprous cyanide (7.57 g, 84.7 mmol) in anhydrous THF (76 mL) at -5 °C under argon was added dropwise allylmagnesium chloride (2.0 M in THF, 46.6 mL, 93.2 mmol). The resulting slurry was stirred for 15 min. at -5 °C and a cold solution of **2** (5.0 g, 14.12 mmol) in THF (200 mL) added dropwise. The reaction mixture was stirred at room temperature for 6 h, quenched by adding 10% $NaHCO_3$ at 0 °C, and diluted with ethyl acetate. The organic layer was washed with 10% $NaHCO_3$ twice, dried ($Na_2SO_4$), and concentrated to give a mixture of **3** and **4** (a white foam, 5.18 g). The two diastereomers (ratio: ~ 1:1) were separated by chromatography on silica with 15% EtOAc in $CHCl_3$.

**3** (higher $R_f$): $^1H$ NMR ($CDCl_3$) δ 0.07 (s, 6H, $SiMe_2$), 0.88 (s, 9H, t-butyl), 1.91 (s, 3H, 5-$CH_3$), 2.14-2.19 (m, 1H, H2'a), 2.30-2.38 (m, 3H, H2'b, $CH_2C$=C), 2.56 (d, 1H, J = 4.5 Hz, 5'-OH), 3.73-3.78 (m, 1H, H4'), 3.80 (t, 1H, J = 2.3 Hz, H5'), 4.47-4.50 (m, 1H, H3'), 5.16 (d, 1H, J = 3.3 Hz, H of -C=$CH_2$), 5.18 (s, 1H, H of -C=$CH_2$), 5.80-5.88 (m, 1H, C-CH=C), 6.17 (t, 1H, J = 6.9 Hz, H1'), 7.52 (s, 1H, H6), 9.00 (s, 1H, NH); FABMS m/z 397 ($MH^+$), 271 ($M^+$-thymine base); Anal. Calcd. for $C_{19}H_{32}N_2O_5Si$: C, 57.54; H, 8.13; N, 7.06. Found: C, 57.54; H, 8.10; N 6.95.

**4** (lower $R_f$): $^1H$ NMR ($CDCl_3$) δ 0.08 (s, 1H, $SiMe_2$), 0.89 (s, 9H, t-butyl), 1.92 (s, 3H, 5-$CH_3$), 2.14-2.19 (m, 1H, H2'a), 2.31-2.39 (m, 3H, H2'b, $CH_2C$=C), 2.43 (d, 1H, J = 5.0 Hz, 5'-OH), 3.75 (dd, 1H, J = 11.4 Hz, 5.3 Hz, H4'), 3.81 (s, 1H, H5'), 4.49 (m, 1H, H3'), 5.17 (d, 1H, J = 6.5 Hz, H of -C=$CH_2$), 5.20 (s, 1H, H of -C=$CH_2$C), 5.80-5.89 (m, 1H, C-CH=C), 6.17 (t, 1H, J = 7.0 Hz, H1'), 7.52 (s, 1H, H6), 8.33 (s, 1H, NH); FABMS m/z 397 ($MH^+$), 271 ($M^+$-thymine base); Anal. Calcd. for $C_{19}H_{32}N_2O_5Si$: C, 57.54; H, 8.13; N, 7.06. Found: C, 57.44; H, 8.05; N, 6.99.



Method B. To a stirred solution of titanium tetraisopropyl orthotitanate (Fluka, 31.15 mL, 0.105 mol) in anhydrous THF (75 mL) at 0 °C under argon was added dropwise titanium tetrachloride (1.0 M in THF, 35.25 mL, 0.035 mol). The resulting mixture was stirred at room temperature for 15 min. and 240 mL of THF added. After cooling to 0 °C, allylmagnesium bromide (1.0 M in ether, 141 mL, 0.141 mol) was added slowly and stirred for 20 min. A solution of **2** (5.0 g, 14.1 mmol) in THF (90 mL) was added dropwise and the resulting reaction mixture stirred at 0 °C for 1 h. The reaction was quenched by adding water (400 mL) and the resulting mixture stirred at room temperature for 30 min. After adding more water (500 mL) and ethyl acetate (500 mL), the mixture was extracted with ethyl acetate twice. The combined organic layer was dried ($Na_2SO_4$) and concentrated. Chromatography on silica (ethyl acetate-hexanes 1:2) yielded a mixture of **3** and **4** (ratio: 1.3:1, 3.52 g, 62.9%) and 135 mg of the intact **2**. The products **3** and **4** obtained from Method B have the identical $R_f$ values and $^1H$ NMR spectra as those from Method A.

**3'-*O*-(t-Butyldimethylsilyl)-5'-deoxy-5'-methylidenethymidine 5**. A suspension of sodium hydride (60% in mineral oil, 2.88 g, 72 mmol) in anhydrous DMSO (100 mL), after stirring at 65 °C for 1.5 h under nitrogen, became a cloudy solution, which was cooled to room temperature and transferred to a flask containing a cold, stirred suspension of methyltriphenylphosphonium bromide (27.0 g, 75.6 mmol) in DMSO (20 mL) under nitrogen. The mixture was stirred at room temperature for 45 min and a solution of **2** (8.50 g, 24 mmol) in DMSO (40 mL) added at 0 °C. The resulting reaction mixture was stirred at room temperature for 2 h, diluted with ethyl acetate, washed with brine five times, dried ($Na_2SO_4$), and concentrated. The crude was purified by

chromatography on silica (EtOAc-hexanes, 30:70) to give 6.79 g (80%) of **5** as a white solid, m.p. 122-3° (recrystallized from ethyl acetate and hexanes). $^1$H NMR (acetone-$d_6$) δ 0.088 (s, 3H, $SiCH_3$), 0.094 (s, 3H, $SiCH_3$), 0.89 (s, 9H, t-butyl), 1.81 (s, 3H, 5-$CH_3$), 2.20-2.26 (m, 1H, H2'a), 2.34-2.39 (m, 1H, H2'b), 4.18 (m, 1H, H4'), 4.37 (m, 1H, H3'), 5.23 (d, 1H, J = 11.0 Hz, H of -C=$CH_2$), 5.36 (m, 1H, H of -C=$CH_2$), 5.99-6.06 (m, 1H, C--CH=C), 6.27 (t, 1H, J = 6.5 Hz, H1'), 7.44 (s, 1H, H6), 10.05 (s, br, 1H, NH); FABMS m/z 353 ($MH^+$), 227 ($M^+$-thymine base); Anal. Calcd. for $C_{17}H_{28}N_2O_4Si$: C, 57.92; H, 8.01; N, 7.95. Found: C, 57.89; H, 8.04; N, 7.83.

**3'-*O*-(t-Butyldimethylsilyl)-5'(*S,R*)-epoxy thymidine derivatives 6 and 7.** A solution of **5** (6.26 g, 17.78 mmol) and m-chloroperoxybenzoic acid (4.61 g, 26.68 mmol) in methylene chloride (160 mL) was stirred at room temperature overnight, diluted with methylene chloride, washed with 10% $NaHCO_3$ twice, then with brine, dried ($Na_2SO_4$), and concentrated. The residue was chromatographed on silica (EtOAc-hexanes, 1:2) to give the intact **5** (2.25 g, 35.9%), **7** (higher $R_f$ isomer, 3.2 g, 76%), and **6** (lower $R_f$ isomer, 0.36 g, 8%), both **6** and **7** as a white solid. **6**: m.p. 120-123° (from EtOAc/hexanes); $^1$H NMR ($CDCl_3$) δ 0.08 (s, 6H, $SiMe_2$), 0.88 (s, 9H, t-butyl), 1.94 (s, 3H, 5-$CH_3$), 2.11-2.17 (m, 1H, H2'a), 2.25-2.31 (m, 1H, H2'b), 2.71 (t, 1H, J = 3.4 Hz, $CH_2$ of epoxy), 2.94 (t, 1H, J = 4.5 Hz, $CH_2$ of epoxy), 3.21 (dd, 1H, J = 6.8 Hz, 3.4 Hz, H5'), 4.05 (t, 1H, J = 3.2 Hz, H4'), 4.19 (m, 1H, H3'), 6.31 (t, 1H, J = 6.7 Hz, H1'), 7.28 (s, 1H, H6), 8.65 (s, 1H, NH); FABMS m/z 369 ($MH^+$), 243 ($M^+$-thymine base); **7**: m.p. 124-125.5° (from EtOAc/hexanes); $^1$H NMR ($CDCl_3$) δ 0.10 (s, 6H, $SiMe_2$), 0.90 (s, 9H, t-butyl), 1.92 (s, 3H, 5-$CH_3$), 2.05-2.10 (m, 1H, H2'a), 2.15-2.20 (m, 1H, H2'b), 2.80-2.84 (m, 2H, $CH_2$ of epoxy), 3.17 (m, 1H, H5'), 4.08 ( m, 1H, H4'), 4.52 (m, 1H, H3'), 6.37 (dd, J = 7.6 Hz, 6.0 Hz, H1'), 7.49 (s, 1H, H6), 8.97 (s, 1H, NH); FABMS m/z 369 ($MH^+$), 243 ($M^+$-thymine base); Anal. Calcd. for $C_{17}H_{28}N_2O_5Si$: C, 55.41; H, 7.66; N, 7.60. Found: C, 55.47; H, 7.43; N, 7.44.

**3'-*O*-(t-Butyldimethylsilyl)-5'(*S*)-*C*-methoxymethylthymidine 8.** A suspension of **7** (2.86 g, 7.77 mmol) and alumina (ICN, neutral, Super I, 2.87 g) in methanol (150 mL) was stirred at 65 °C for 5 days. The slurry was loaded on a short silica column and the product eluted with methanol. The methanol solution was concentrated and the crude purified by chromatography on silica (EtOAc-hexanes 1:1) to give 2.36 g (76%) of **8** as a white solid; $^1$H NMR ($CDCl_3$) δ 0.06 (s, 6H, $SiMe_2$), 0.86 (s, 9H, t-butyl), 1.89 (s, 3H, 5-$CH_3$), 2.13-2.27 (m, 2H, H2'a and H2'b), 3.43 (s, 3H, $OCH_3$), 3.47-3.55 (m, 2H, $CH_2OMe$), 3.64 (d, 1H, J = 3.7 Hz, 5'-OH), 3.81 (s, 1H, H5'), 3.96 (t, 1H, J = 4.2 Hz, H4'), 4.52 (m, 1H, H3'), 6.27 (t, 1H, J = 6.6 Hz, H1'), 7.73 (s, 1H, H6), 9.51 (s, 1H, NH); FABMS m/z 401 ($MH^+$), 275 ($M^+$-thymine base); Anal. Calcd. for $C_{18}H_{32}N_2O_6Si$: C, 53.97; H, 8.05; N, 6.99. Found: C, 53.92; H, 7.93; N, 6.81.

**3'-*O*-(t-Butyldimethylsilyl)-5'(*S*)-*C*-trifluoroacetamidomethylthymidine 10**. A solution of **7** (0.84 g, 2.28 mmol) in methanol (5 mL) was mixed with ammonia-saturated methanol solution (10 mL). The resulting solution stood at room temperature overnight and the excess ammonia and methanol evaporated. The dried crude **9** was dissolved in dioxane (10 mL) and S-ethyl thiotrifluoroacetate (1.80 g, 11.4 mmol, 1.46 mL) added. The resulting solution was stirred at room temperature for 6 h and concentrated. The residue was chromatographed on silica (EtOAc-hexanes, 1:1) to give 895 mg (81.8%) of **10** as a white solid; $^1$H NMR ($CDCl_3$) δ 0.08 (s, 6H, $SiMe_2$), 0.88 (s, 9H, t-butyl), 1.90 (s, 3H, 5-$CH_3$), 2.04-2.08 (m, 1H, H2'a), 2.70-2.76 (m, 1H, H2'b), 3.20-3.26 (m, 1H, H of $CH_2N$), 3.73-3.78 (m, 1H, H of $CH_2N$), 3.86 (m, 1H, H4'), 3.95 (s, 1H, H5'), 4.27 (br, 1H, 5'-OH), 4.54 (m, 1H, H3'), 6.18 (dd, 1H, J = 8.7 Hz, 5.9 Hz, H1'), 7.21 (s, 1H, H6), 8.00 (t, br, 1H, $HNCOCF_3$), 10.19 (s, 1H, NH of thymine); FABMS m/z 482 ($MH^+$); Anal. Calcd. for $C_{19}H_{30}F_3N_3O_6Si$: C, 47.39; H, 6.28; N, 8.73. Found: C, 47.53; H, 6.23; N, 8.58.

**3'-*O*-(t-Butyldimethylsilyl)-5'(*S*)-*C*-cyanomethylthymidine 11**. A mixture of **7** (0.77 g, 2.09 mmol) and potassium cyanide (520 mg, 8.0 mmol) in DMF (10 mL) was stirred at room temperature for 40 h, diluted with ethyl acetate, washed with brine five times, dried ($Na_2SO_4$), and concentrated. The crude was purified by chromatography on silica (EtOAc-hexanes, 1:1) to give **11** (580 mg, 70%) as a white solid, m.p. 158-159° (from EtOAc/hexanes); $^1$H NMR ($CDCl_3$) δ 0.09 (s, 6H, $SiMe_2$), 0.89 (s, 9H, t-butyl), 1.93 (s, 3H, 5-$CH_3$), 2.10-2.14 (m, 1H, H2'a), 2.56-2.72 (m, 3H, H2'b, $CH_2CN$), 3.95 (s, 1H, H5'), 4.04-4.09 (m, 1H, H4'), 4.27 (d, 1H, J = 9.0 Hz, 5'-OH), 4.56 (m, 1H, H3'), 5.85 (t, 1H, J = 7.0 Hz, H1'), 7.23 (s, 1H, H6), 9.06 (s, 1H, NH); FABMS m/z 396 ($MH^+$), 270 ($M^+$ - thymine base); Anal. Calcd. for $C_{18}H_{29}N_3O_5Si$: C, 54.66; H, 7.39; N, 10.62. Found: C, 54.35; H, 7.10; N, 10.30.

**3'-*O*-(t-Butyldimethylsilyl)-5'(*S*)-*C*-bromomethylthymidine 12.** To a stirred solution of **7** (1.53 g, 4.15 mmol) in anhydrous THF (30 mL) at 0 °C under nitrogen was added methylmagnesium bromide (1.4 M in toluene-THF, Aldrich, 8.9 mL, 12.45 mmol) dropwise during 10 min. The resulting solution was stirred at 0 °C for 90 min. and 2 mL of 5% AcOH added dropwise. The resulting mixture was stirred for 10 min at 0 °C, diluted with ethyl acetate, washed with 5% AcOH, with water, with 5% $NaHCO_3$, dried ($Na_2SO_4$), and concentrated. The crude showed only one spot on TLC (silica, EtOAc-hexanes 1:1). The white solid **12** weighed 1.73 g; $^1$H NMR ($CDCl_3$) δ 0.09 (s, 6H, $SiMe_2$), 0.89 (s, 9H, t-butyl), 1.92 (s, 3H, 5-$CH_3$), 2.11-2.15 (m, 1H, H2'a), 2.48-2.54 (m, 1H, H2'b), 3.48 (d, 2H, J = 7.2 Hz, $CH_2Br$), 3.51 (d, 1H, J = 7.0 Hz, 5'-OH), 3.94 (m, 1H, H4'), 4.09 (m, 1H, H5'), 4.54 (m, 1H, H3'), 6.04 (t, 1H, J = 7.1 Hz, H1'), 7.39 (s, 1H, H6); FABMS m/z 451 ($MH^+$), 449 ($MH^+$), 325 ($MH^+$-thymine), 323 ($MH^+$-thymine); Anal. Calcd. for $C_{17}H_{29}BrN_2O_5Si$: C, 45.43; H, 6.51; N, 6.23. Found: C, 45.64; H, 6.41; N, 6.06.

**5'-*O*-(4,4'-Dimethoxytrityl)-5'(*S*)-*C*-methoxymethylthymidine 13**. A mixture of **8** (140 mg, 0.35 mmol), 4,4'-dimethoxytrityl chloride (DMT-Cl, 600 mg, 1.75 mmol), and silver triflate (AgOTf, 450 mg, 1.75 mmol) in anhydrous pyridine (1.5 mL) was heated at 55 °C for 3 days. Precipitates were filtered and washed with pyridine. The filtrate was concentrated and the residue chromatographed on silica (ethyl acetate-hexanes, 1:1) to give 206 mg (83%) of 5'-*O*-DMT product as a foam; FABMS m/z 703 ($MH^+$), 702 ($M^+$), 303 (DMT).

The 5'-*O*-DMT product was dissolved in THF (3 mL) and TBAF (1.0 M in THF, 1.0 mL) added. The resulting solution stood at room temperature for 1 h. Solvent was evaporated and the residue chromatographed on silica (ethyl acetate-hexanes, 70:30) to give 152 mg of **13** as a colorless foam; $^1H$ NMR ($CDCl_3$) δ 1.84 (s, 3H, 5-$CH_3$), 2.19-2.25 (m, 1H, H2'a), 2.30-2.36 (m, 2H, H2'b, 3'-OH), 3.17 (s, 3H, $OCH_3$), 3.21 (d, 2H, J = 4.8 Hz, $CH_2OMe$), 3.68 (dd, 1H, J = 9.8 Hz, 5.0 Hz, H4'), 3.79, 3.80 (2s, 6H, 2Ar$OCH_3$), 3.86 (t, 1H, J = 5.0 Hz, H5'), 4.51 (m, 1H, H3'), 6.25 (t, 1H, J = 6.6 Hz, H1'), 6.81-6.85 (m, 4H, DMT), 7.16-7.48 (m, 10 H, DMT, H6), 8.24 (s, 1H, NH); FABMS m/z 589 ($MH^+$), 588 ($M^+$), 303 (DMT).

**5'-*O*-(4,4'-Dimethoxytrityl)-5'(*S*)-*C*-trifluoroacetamidomethylthymidine 14.** A mixture of **10** (392 mg, 0.815 mmol), DMT-Cl (1.38 g, 4.07 mmol), and AgOTf (1.05 g, 4.07 mmol) in pyridine (3 mL) was heated at 50 °C for 6 days. The same work-up and purification procedures as described for **13** gave 570 mg of the 5'-*O*-DMT product as a foam and 175 mg of the intact **10**. FABMS m/z 784 ($MH^+$), 783 ($M^+$), 303 (DMT).

The 5'-*O*-DMT product was dissolved in THF (6 mL) and TBAF (1.0 M in THF, neutralized with AcOH, pH 8.0, 2.0 mL) added. The solution stood at room temperature overnight. Solvent was evaporated and the residue chromatographed on silica (ethyl acetate-hexanes, 3:1) to give 214 mg of **14** as a colorless foam; $^1H$ NMR ($CDCl_3$) δ 1.90 (s, 3H, 5-$CH_3$), 2.14 (d, 1H, J = 3.6 Hz, 3'-OH), 2.28 (t, 2H, J = 6.3 Hz, H2'a, H2'b), 3.04-3.09 (m, 1H, H of $CH_2N$), 3.40-3.45 (m, 1H, H of $CH_2N$), 3.77 (t, 1H, J = 4.7 Hz, H4'), 3.80 (s, 6H, $2CH_3O$), 3.89-3.93 (m, 1H, H5'), 4.38-4.43 (m, 1H, H3'), 6.16 (t, 1H, J = 6.9 Hz, H1'), 6.74 (t, 1H, J = 5.7 Hz, $HNCOCF_3$), 6.83-6.87 (m, 4H, DMT), 7.24-7.46 (m, 9H, DMT), 8.40 (s, 1H, NH of thymine); FABMS m/z 670 ($MH^+$), 669 ($M^+$), 303 (DMT).

**5'(*S*)-*C*-Allyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 15.** A mixture of **3** (1.20 g, 1.72 mmol), DMT-Cl (2.91 g, 8.60 mmol), and AgOTf (2.21 g, 8.60 mmol) in pyridine (10 mL) was stirred at 55 °C for 6 days. The same work-up and purification procedures as described for **13** gave 1.31 g of the 5'-*O*-DMT product as a foam; FABMS m/z 699 ($MH^+$), 698 ($M^+$), 303 (DMT).

The 5'-*O*-DMT product (450 mg, 0.64 mmol) was dissolved in THF (6 mL) and TBAF (1.0 M in THF, 1.92 mL) added. The solution stood at room temperature for 2 h. Solvent was evaporated and the residue chromatographed on silica (ethyl acetate-hexanes, 2:1) to give 351 mg of **15** as a colorless foam; $^1$H NMR ($CDCl_3$) δ 1.84 (s, 3H, 5-$CH_3$), 1.95 (d, 1H, J = 3.9 Hz, 3'-OH), 2.19-2.44 (m, 4H, H2'a, H2'b, $CH_2C$=C), 3.42 (m, 1H, H4'), 3.80 (m, 7H, H5', 2$CH_3O$), 4.39 (m, 1H, H3'), 4.89 (d, 1H, J = 16.2 Hz, H of -C=$CH_2$), 4.92 (d, 1H, J = 6.8 Hz, H of -C=$CH_2$), 5.36-5.45 (m, 1H, C-CH=C), 6.33 (t, 1H, J = 6.7 Hz, H1'), 6.81-6.86 (m, 4H, DMT), 7.21-7.47 (m, 9H, DMT), 7.87 (s, 1H, H6), 8.72 (s, 1H, NH); FABMS m/z 585 ($MH^+$), 584 ($M^+$), 303 (DMT); Anal. Calcd. for $C_{34}H_{36}N_2O_7$: C, 69.84; H, 6.21; N, 4.79. Found: C,; H,; N,.

**5'(*R*)-*C*-Allyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 27.** A mixture of **4** (439 mg, 1.1 mmol), DMT-Cl (1.12 g, 3.3 mmol), and AgOTf (858 mg, 3.3 mmol) in pyridine (4 mL) was stirred at 50 °C for 20 h. The same work-up and purification procedures as described for **13** gave 660 mg (85%) of **25** as a foam; FABMS m/z 699 ($MH^+$), 698 ($M^+$), 303 (DMT).

416 mg (0.596 mmol) of **25** was dissolved in THF (6 mL) and TBAF (1.0 M in THF, 2.4 mL) added. The resulting solution stood at room temperature for 1 h. Solvent was evaporated and the residue chromatographed on silica (ethyl acetate-hexanes, 2:1) to give 287 mg of **27** as a colorless foam; $^1$H NMR ($CDCl_3$) δ 1.47 (s, 3H, 5-$CH_3$), 2.08-2.33 (m, 5H, H2'a', H2'b, $CH_2C$=C, 3'-OH), 3.37 (m, 1H, H4'), 3.780, 3.788 (2s, 6H, 2$CH_3O$), 3.89 (t, 1H, J = 3.5 Hz, H5'), 4.70 (m, 1H, H3'), 4.91 (d, 1H, J = 17.6 Hz, H of -C=$CH_2$), 4.96 (d, 1H, J = 10.1 Hz, H of -C=$CH_2$), 5.48-5.57 (m, 1H, C-CH=C), 6.26 (dd, 1H, J = 8.0 Hz, 5.8 Hz, H1'), 6.82-6.86 (m, 4H, DMT), 6.95 (s, 1H, H6), 7.20-7.50 (m, 9H, DMT), 8.54 (s, 1H, NH); FABMS m/z 585 ($MH^+$), 584 ($M^+$), 303 (DMT); Anal. Calcd. for $C_{34}H_{36}N_2O_7$: C, 69.84; H, 6.21; N, 4.79. Found: C,; H,; N,.

**5'(*S*)-*C*-Acetoxymethyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 16.** To a mixture of DMT-Cl (3.72 g, 11.0 mmol) and AgOTf (2.83 g, 11.0 mmol) in pyridine (5 mL) was added a solution of **12** (1.64 g, 3.65 mmol) in pyridine (5 mL). The resulting mixture was stirred at 45 °C for 24 h. The same work-up and purification procedures as described for **13** yielded 1.92 g (70%) of the 5'-*O*-DMT product as a foam; FABMS m/z 752 ($M^+$), 750 ($M^+$), 303 (DMT).

The 5'-*O*-DMT product (2.58 g, 3.44 mmol) and anhydrous sodium acetate (2.82 g, 34.4 mmol) in anhydrous DMF (32 mL) was stirred at 95-100 °C for 4 days. The mixture was diluted with ethyl acetate, washed with water five times, dried ($Na_2SO_4$), and concentrated. Chromatography on silica (EtOAc-hexanes, 1:1) gave 1.52 g (60%) of the acetoxy derivative as a colorless foam.

A solution of the 5'-acetoxymethyl-5'-*O*-DMT derivative (480 mg, 0.656 mmol) and TBAF (1.0 M in THF, neutralized with AcOH to pH 7.0, 2.6 mL) in THF (10 mL) stood at room temperature overnight. Solvent was evaporated and the residue chromatographed on silica with ethyl acetate as eluent to give 329 mg (81%) of **16** as a colorless foam; $^1$H NMR ($CDCl_3$) δ 1.87 (s, 3H, 5-$CH_3$), 1.93 (s, 3H, $CH_3CO$), 2.17-2.23 (m, 1H, H2'a), 2.29-2.34 (m, 1H, H2'b), 3.74-3.84 (m, 8H, H4', H5', 2$OCH_3$), 4.02 (dd, 1H, J = 11.3 Hz, 6.7 Hz, H of $CH_2OAc$), 4.15 (dd, 1H, J = 11.3 Hz, 3.8 Hz, H of $CH_2OAc$), 4.40 (br, 1H, H3'), 6.25 (t, 1H, J = 6.8 Hz, H1'), 6.79-6.88 (m, 4H, DMT), 7.23-7.47 (m, 9H, DMT), 7.59 (s, 1H, H6), 8.65 (s, 1H, NH), FABMS m/z 639 ($MNa^+$), 616 ($M^+$), 303 (DMT).

**5'-*O*-(4,4'-Dimethoxytrityl)-5'(*S*)-*C*-[(2,4-dinitrophenyl)imidazole-4-acetamido]methylthymidine 17.** A solution of **14** (160 mg, 0.24 mmol) in methanol (2 mL) and 30% $NH_4OH$ (2 mL) stood at room temperature overnight. Ammonia and solvents were evaporated and the residue coevaporated with ethanol. The dried, crude product was dissolved in a solution of 1-(2,4-dinitrophenyl)imidazole-4-acetic acid succinimide ester[18] (112 mg, 0.29 mmol) in THF (5 mL). The resulting solution stood at room temperature for 5 h. THF was evaporated and the residue chromatographed on silica with 10% methanol in methylene chloride to give 149 mg of **17** as a yellow solid; $^1$H NMR (acetone-$d_6$) δ 1.77 (s, 3H, 5-$CH_3$), 2.12-2.17 (m, 1H, H2'a), 2.25-2.30 (m, 1H, H2'b), 3.47 (s, 2H, $CH_2$-imidazole), 3.54-3.59 (m, 1H, H of $NCH_2$), 3.75 (s, 6H, 2$OCH_3$), 3.76-3.81 (m, 2H, H5', H of $NCH_2$), 3.93 (dd, 1H, J = 4.8 Hz, 3.3 Hz, H4'), 4.52 (m, 1H, H3'), 6.25 (dd, 1H, J = 8.1 Hz, 6.2 Hz, H1'), 6.84-6.87 (m, 4H, DMT), 7.18-7.29 (m, 3H, DMT), 7.34 (m, 2H, 2NHCO), 7.44-7.59 (m, 6H, DMT), 7.73 (s, 1H, imidazole), 7.90 (d, 1H, J = 1.0 Hz, imidazole), 7.97 (d, 1H, J = 8.7 Hz, DNP), 8.01 (s, 1H, H6), 8.70 (dd, 1H, J = 8.7 Hz, 2.7 Hz, DNP), 8.95 (d, 1H, J = 2.5 Hz, DNP), FABMS m/z 848 ($MH^+$), 303 (DMT).

**5'(*S*)-*C*-Cyanomethyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 18.** A mixture of **11** (438 mg, 1.22 mmol), DMT-Cl (2.06 g, 6.11 mmol), and AgOTf (1.57 g, 6.11 mmol) in pyridine (6 mL) was stirred at 50 °C for 3 days. The same work-up and purification procedures as described for **13** gave 705 mg of the 5'-*O*-DMT product as a yellow powder; FABMS m/e 698 ($MH^+$), 697 ($M^+$), 303 (DMT).

The 5'-*O*-DMT product (665 mg, 0.954 mmol) was dissolved in THF (10 mL) and TBAF (1.0 M in THF, 3.7 mL) added. The solution stood at room temperature for 1 h. Solvent was evaporated and the residue chromatographed on silica (ethyl acetate-hexanes, 3:1) to give 445 mg of pure **18** as a colorless powder; $^1$H NMR ($CDCl_3$) δ 1.88 (s, 3H, 5-$CH_3$), 2.03-2.09 (m, 1H, H2'a), 2.16 (d, 1H, J = 3.1 Hz, 3'-OH), 2.28-2.38 (m, 3H, H2'b, $CH_2CN$), 3.81 (s, 6H, 2$CH_3O$), 3.92 (t, 1H, J = 5.0 Hz, H4'), 4.02 (m, 1H, H5'), 4.77 (m, 1H, H3'), 6.23 (t, 1H, J = 7.0 Hz, H1'), 6.82-6.88 (m, 4H, DMT), 7.16-7.40 (m, 7H,

DMT), 7.42 (s, 1H, H6), 7.46 (d, 2H, J = 7.5 Hz, DMT), 8.45 (s, 1H, NH); FABMS m/z 584 ($MH^+$), 583 ($M^+$), 303 (DMT); Anal. Calcd. for $C_{33}H_{33}N_3O_7$: C, 67.91; H, 5.70; N, 7.20. Found: C,; H,; N,.

**5'(*R*)-*C*-Acetoxypropyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 28.** To a stirred solution of borane methylsulfide (2.0 M in THF, 6.15 mL, 12.31 mmol) in anhydrous THF (4 mL) at 0 °C under argon was added dropwise **25** (1.43 g, 2.05 mmol) in THF (12 mL). The resulting reaction mixture was stirred at 0 °C for 3 h, quenched by slowly adding methanol (23 mL), water (23 mL), and sodium perborate (1.89 g, 12.31 mmol). The resulting mixture was stirred at room temperature for 2 h, the insoluble solid filtered, and the filtrate concentrated. The residue was dissolved in ethyl acetate, washed with brine three times, dried ($Na_2SO_4$), and concentrated. Chromatography on silica (EtOAc-hexanes 1:1) gave 0.82 g (56%) of the 5'-hydroxypropyl product as a white foam.

A solution of the 5'-hydroxypropyl product (326 mg, 0.455 mmol) in acetic anhydride (0.5 mL) and pyridine (3.5 mL) stood at r.t. for 2 h, quenched by adding methanol (0.5 mL) and water (0.5 mL), stirred at r.t. for 1 h, diluted with ethyl acetate, washed with 5% $NaHCO_3$ three times, dried ($Na_2SO_4$), and concentrated. The residue was dissolved in THF (3 mL) and TBAF (1.0 M, 1.3 mL) added. The solution stood at r.t. for 10 min, diluted with ethyl acetate, washed with 5% $NaHCO_3$ three times, dried ($Na_2SO_4$), and concentrated. Chromatography on silica (EtOAc-hexanes 3:1) gave 237 mg (80.8%) of **28** as a colorless foam); $^1H$ NMR ($CDCl_3$) δ 1.48-1.75 (m, 4H, $2CH_2$), 1.57 (s, 3H, 5-$CH_3$), 1.95 (s, 3H, Ac), 2.02-2.08 (m, 1H, H2'a), 2.24-2.29 (m, 1H, H2'b), 2.44 (m, 1H, 3'-OH), 3.33 (m, 1H, H4'), 3.781, 3.788 (2s, 6H, $2OCH_3$), 3.81-3.86 (m, 3H, H5', $CH_2O$), 4.45 (m, 1H, H3'), 6.23 (dd, 1H, J = 7.3 Hz, 6.4 Hz, H1'), 6.82-6.85 (m, 4H, DMT), 6.87 (s, 1H, H6), 7.19-7.48 (m, 9H, DMT), 8.54 (s, 1H, NH).

**5'(*S*)-*C*-Allyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 21.** To a stirred solution of **15** (238 mg, 0.40 mmol) and diisopropylethylamine (0.28 mL, 1.6 mmol) in $CH_2Cl_2$ (anhydrous, 3 mL) at 0 °C under nitrogen was added dropwise a solution of 2-cyanoethyl-N,N-diisopropyl-chlorophosphoramidite (189 mg, 0.18 mL, 0.8 mmol) in $CH_2Cl_2$ (1 mL). The resulting solution was stirred at room temperature for 30 min, diluted with ethyl acetate at 0 °C, washed with cold 5% $NaHCO_3$ three times, dried ($Na_2SO_4$), and concentrated at room temperature. Chromatography on silica with $Et_3N$-EtOAc-hexanes (5:45:50) as eluent gave **21** (two diastereomers, 240 mg, 75%) as a colorless foam; the higher $R_f$ diastereomer: $^1H$ NMR ($CDCl_3$) δ 1.11 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.14 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.76 (s, 3H, 5-$CH_3$), 2.17-2.46 (m, 4H, H2'a, H2'b, $CH_2C$=C), 2.28 (t, 2H, J = 6.7 Hz, $CH_2CN$), 3.32-3.57 (m, 5H, H4', $2NCHMe_2$, $POCH_2$), 3.803, 3.807 (2s, 6H, $2OCH_3$), 4.02 (s, 1H, H5'), 4.50 (m, 1H, H3'), 4.86 (d, 1H, J = 17.3 Hz, H of -C=$CH_2$),

4.93 (d, 1H, J = 10.2 Hz, H of -C=$CH_2$), 5.30-5.40 (m, 1H, C-CH=C), 6.35 (t, 1H, J = 6.3 Hz, H1'), 6.82-6.87 (m, 4H, DMT), 7.22-7.47 (m, 9H, DMT), 7.99 (s, 1H, H6), 8.49 (s, 1H, NH); $^{31}$P ($CDCl_3$) δ 150.0; FABMS m/z 886 ($MHEt_3N^+$), 785 ($MH^+$), 303 (DMT); the lower $R_f$ diastereomer: $^1$H NMR ($CDCl_3$) δ 1.11 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.14 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.76 (s, 3H, 5-$CH_3$), 2.17-2.46 (m, 4H, H2'a, H2'b, $CH_2C$=C), 2.28 (t, 2H, J = 6.7 Hz, $CH_2CN$), 3.32-3.57 (m, 5H, H4', 2NCHMe$_2$, $POCH_2$), 3.803, 3.807 (2s, 6H, 2$OCH_3$), 4.02 (s, 1H, H5'), 4.50 (m, 1H, H3'), 4.86 (d, 1H, J = 17.3 Hz, H of -C=$CH_2$), 4.93 (d, 1H, J = 10.2 Hz, H of -C=$CH_2$), 5.30-5.40 (m, 1H, C-CH=C), 6.35 (t, 1H, J = 6.3 Hz, H1'), 6.82-6.87 (m, 4H, DMT), 7.22-7.47 (m, 9H, DMT), 7.99 (s, 1H, H6), 8.49 (s, 1H NH); $^{31}$P ($CDCl_3$) δ 149.2.

**5'(*R*)-*C*-Allyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 29.** The procedure as described for **21** gave 279 mg (88%) of **29** as a colorless foam from 238 mg (0.40 mmol) of **27**; the higher $R_f$ diastereomer: $^1$H NMR ($CDCl_3$) δ 1.25 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.27 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.33 (s, 3H, 5-$CH_3$), 2.17-2.28 (m, 3H, H2'a, H2'b, H of $CH_2C$=C), 2.43 (dd, 1H, J = 13.2 Hz, 5.1 Hz, H of $CH_2C$=C), 2.65 (t, 1H, J = 5.8 Hz, $CH_2CN$), 3.28-3.32 (m, 1H, H4'), 3.63-3.71 (m, 2H, $POCH_2$), 3.76-3.91 (m, 8H, 2NCHMe$_2$, 2$CH_3O$), 4.03 (s, 1H, H5'), 4.87 (d, 1H, J = 15.9 Hz, H of -C=$CH_2$), 4.89 (d, 1H, J = 8.6 Hz, H of -C=$CH_2$), 5.08 (m, 1H, H3'), 5.25-5.33 (m, 1H, C-CH=C), 6.28 (dd, 1H, J = 9.4 Hz, 5.1 Hz, H1'), 6.81-6.86 (m, 4H, DMT), 7.04 (s, 1H, H6), 7.18-7.51 (m, 9H, DMT), 8.21 (s, 1H, NH); $^{31}$P ($CDCl_3$) δ 148.2; the lower $R_f$ diastereomer: $^1$H NMR ($CDCl_3$) δ 1.21 (d, 12H, J = 6.2 Hz, 2$CMe_2$), 1.37 (s, 3H, 5-$CH_3$), 2.08-2.25 (m, 3H, H2'a, H2'b, H of $CH_2C$=C), 2.52 (dd, 1H, J = 13.6 Hz, 5.6 Hz, H of $CH_2C$=C), 2.68 (t, 2H, J = 5.5 Hz, $CH_2CN$), 3.30-3.34 (m, 1H, H4'), 3.60-3.68 (m, 2H, $POCH_2$), 3.71-3.79 (m, 7H, NCHMe$_2$, 2$CH_3O$), 3.91-3.98 (m, 1H, NCHMe$_2$), 3.99 (t, 1H, J =2.0 Hz, H5'), 4.82 (d, 1H, J = 17.0 Hz, H of -C=$CH_2$), 4.88 (d, 1H, J = 10.0 Hz, H of -C=$CH_2$), 5.09 (m, 1H, H3'), 5.29-5.33 (m, 1H, C-CH=C), 6.25 (dd, 1H, J = 9.1 Hz, 5.3 Hz, H1'), 6.81-6.85 (m, 4H, DMT), 7.02 (s, 1H, H6), 7.18-7.51 (m, 9H, DMT), 8.51 (s, 1H, NH); $^{31}$P ($CDCl_3$) δ 149.2.

**5'-*O*-(4,4'-Dimethoxytrityl)-5'(*S*)-*C*-methoxymethylthymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 19.** The procedure as described for **21** gave 584 mg (89%) of **19** as a colorless foam from 490 mg (0.83 mmol) of **13**; $^1$H NMR (two diastereomers, $CD_3CN$) δ 1.03-1.16 (m, 12H, 2$CMe_2$), 1.71 (s, 3H, 5-$CH_3$), 2.24-2.62 (m, 4H, H2'a, H2'b, $CH_2CN$), 3.01, 3.03 (2s, 3H, $OCH_3$), 3.09-3.27 (m, 2H, $CH_2O$), 3.46-3.70 (m, 5H, H4', 2NCHMe$_2$, $POCH_2$), 3.76 (m, 6H, 2Ar$OCH_3$), 4.07 (m, 1H, H5'), 4.57 (m, 1H, H3'), 6.14 (m, 1H, H1'), 6.84-6.89 (m, 4H, DMT), 7.20-7.48 (m, 9H, DMT), 7.68 (d, 0.5H, J = 1.1 Hz, H6), 7.71 (s, 0.5H, H6), 9.13 (s, 1H, NH); $^{31}$P (two diastereomers, $CD_3CN$) δ 148.6, 149.1; FABMS m/z 890 ($MHEt_3N^+$), 789 ($MH^+$), 303 (DMT).

**5'-*O*-(4,4'-dimethoxytrityl)-5'(*S*)-*C*-trifluoroacetamidomethylthymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 20.** The procedure as described for **21** gave 178 mg (84%) of **20** as a colorless foam from 158 mg (0.268 mmol) of **14**; the higher $R_f$ diastereomer: $^1$H NMR (acetone-$d_6$) δ 1.12 (d, 6H, J = 6.8 Hz, $CMe_2$), 1.16 (d, 6H, J = 6.8 Hz, $CMe_2$), 1.73 (s, 3H, 5-$CH_3$), 2.38-2.54 (m, 4H, H2'a, H2'b, $CH_2CN$), 3.30-3.48 (m, 2H, $2NCHMe_2$), 3.54-3.62 (m, 4H, $POCH_2$, $CH_2NCO$), 3.795, 3.799 (2s, 6H, $2OCH_3$), 3.80-3.86 (m, 1H, H4'), 4.08 (m, 1H, H5'), 4.51 (m, 1H, H3'), 6.25 (dd, 1H, J = 8.0 Hz, 6.0 Hz, H1'), 6.90-6.95 (m, 4H, DMT), 7.24-7.56 (m, 9H, DMT), 7.79 (s, 1H, H6), 8.22 (br, 1H, $NHCOCF_3$), 10.09 (s, 1H, NH); $^{31}$P (acetone-$d_6$) δ 150.2; FABMS m/z 971 ($MH\text{-}Et_3N^+$), 870 ($MH^+$), 303 (DMT); the lower $R_f$ diastereomer: $^1$H NMR (acetone-$d_6$) δ 1.00 (d, 6H, J = 6.8 Hz, $CMe_2$), 1.13 (d, 6H, J = 6.8 Hz, $CMe_2$), 1.72 (s, 3H, 5-$CH_3$), 2.46-2.49 (m, 2H, H2'a, H2'b), 2.73 (t, 2H, J = 6.8 Hz. $CH_2CN$), 3.23-3.29 (m, 1H, $NCHMe_2$), 3.45-3.50 (m, 1H, $NCHMe_2$), 3.51-3.78 (m, 4H, $POCH_2$, $CH_2NCO$), 3.789, 3.793 (2s, 6H, $2OCH_3$), 3.82 (m, 1H, H4'), 4.03 (m, 1H, H5'), 4.49 (m, 1H, H3'), 6.26 (dd, 1H, J = 7.0 Hz, H1'), 6.89-6.93 (m, 4H, DMT), 7.24-7.53 (m, 9H, DMT), 7.77 (s, 1H, H6), 8.28 (br, 1H, $NHCOCF_3$), 10.10 (s, 1H, NH); $^{31}$P (acetone-$d_6$) 150.5.

**5'(*S*)-*C*-Acetoxymethyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 22.** The procedure as described for **21** gave 323 mg (88%) of **22** as a colorless foam from 277 mg (0.45 mmol) of **16**, the higher $R_f$ diastereomer: $^1$H NMR (acetone-$d_6$) δ 1.15 (d, 6H, J = 5.3 Hz, $CMe_2$), 1.17 (d, 6H, J = 7.0 Hz, $CMe_2$), 1.75 (d, 3H, J = 1.4 Hz, 5-$CH_3$), 1.89 (s, 3H, $CH_3CO$), 2.40-2.43 (m, 2H, $CH_2CN$), 2.50-2.60 (m, 2H, H2'a, H2'b), 3.58-3.76 (m, 5H, H4', $POCH_2$, $2NCHMe_2$), 3.79, 3.80 (2s, 6H, $2OCH_3$), 3.92 (dd, 1H, J = 11.6 Hz, 7.2 Hz, H of $CH_2OAc$), 4.07 (dd, 1H, J = 11.6 Hz, 3.9 Hz, H of $CH_2OAc$), 4.12 (m, 1H, H5'), 4.57-4.62 (m, 1H, H3'), 6.26 (t, 1H, J = 6.9 Hz, H1'), 6.90-6.95 (m, 4H, DMT), 7.23-7.55 (m, 9H, DMT), 7.75 (d, 1H, J = 1.4 Hz, H6); $^{31}$P (acetone-$d_6$) δ 150.23; FABMS m/z 817 ($MH^+$), 303 (DMT); the lower $R_f$ diastereomer: $^1$H NMR (acetone -$d_6$) δ 1.15 (d, 6H, J = 5.1 Hz, $CMe_2$), 1.17 (d, 6H, J = 6.8 Hz, $CMe_2$), 1.75 (d, 3H, J = 1.1 Hz, 5-$CH_3$), 1.88 (s, 3H, $CH_3CO$), 2.36-2.42 (m, 1H, H2'a), 2.48-2.52 (m, 1H, H2'b), 2.74 (t, 2H, J = 5.9 Hz, $CH_2CN$), 3.57-3.88 (m, 12H, H4', 1H of $CH_2OAc$, $POCH_2$, $2NCHMe_2$, $2OCH_3$), 4.07 (dd, 1H, J = 11.5 Hz, 3.9 Hz, H of $CH_2OAc$), 4.09 (m, 1H, H5'), 4.61-4.65 (m, 1H, H3'), 6.28 (q, 1H, J = 8.6 Hz, 5.6 Hz, H1'), 6.89-6.93 (m, 4H, DMT), 7.23-7.54 (m, 9H, DMT), 7.70 (d, 1H, J = 1.4 Hz, H6); $^{31}$P (acetone-$d_6$) δ 150.47.

**5'-*O*-(4,4'-Dimethoxytrityl)-5'(*S*)-*C*-[4-(2,4-dinitrophenyl)imidazoleacetamido]-methylthymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphor-amidite) 23.** The procedure as described for **21** gave 84 mg (54%) of **23** as a yellow solid from 127 mg (0.15 mmol) of **17**; $^1$H NMR (two diastereomers, acetone-$d_6$) δ 1.11-1.19 (m, 12H,

$2CMe_2$), 1.75, 1.77 (2s, 3H, 5-$CH_3$), 2.15-2.44 (m, 2H, H2'a, H2'b), 2.57 (t, 1H, J = 6.0 Hz, H of $CH_2CN$), 2.79 (dd, 1H, J = 10.0 Hz, 6.0 Hz, H of $CH_2CN$), 3.45-3.98 (m, 15H, $2NCHMe_2$, $POCH_2$, $CH_2$-imidazole, H4', $2OCH_3$, $CH_2NCO$), 4.12 (m, 1H, H5'), 4.87, 5.00 (2m, 1H, H3'), 6.25-6.32 (m, 1H, H1'), 6.86-6.93 (m, 4H, DMT), 7.20-7.52 (m, 11H, NHCO, CH of imidazole, DMT), 7.88-7.92 (m, 2H, H6, CH of imidazole), 7.94, 7.99 (2d, 1H, J = 8.8 Hz, DNP), 8.69-8.72 (m, 1H, DNP), 8.94 (m, 1H, DNP); $^{31}P$ (acetone-$d_6$) δ 148.75, 148.94; FABMS m/z 1070 ($MNa^+$), 1048 ($MH^+$), 303 (DMT).

**5'(*S*)-*C*-Cyanomethyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 24.** The procedure as described for **21** gave 437 mg (84%) of **24** as a white powder from 388 mg (0.664 mmol) of **18**. $^1H$ NMR (higher $R_f$ diastereomer, acetone-$d_6$) 1.18 (d, 12H, J = 6.6 Hz, $2CMe_2$), 1.78 (s, 3H, 5-$CH_3$), 2.02-2.07 (m, 1H, H of 5'-$CH_2CN$), 2.45-2.55 (m, 3H, H2'a, H2'b, H of 5'-$CH_2CN$), 2.62 (t, 2H, J = 6.0 Hz, $CH_2CN$), 3.61-3.78 (m, 4H, $POCH_2$, $2NCHMe_2$), 3.79, 3.80 (2s, 6H, $2OCH_3$), 4.01 (m, 1H, H5'), 4.22 (t, 1H, J = 4.2 Hz, H4'), 4.58 (m, 1H, H3'), 6.25 (t, 1H, J = 7.0 Hz, H1'), 6.90-6.95 (m, 4H, DMT), 7.24-7.59 (m, 9H, DMT), 7.64 (s, 1H, H6), 10.07 (s, 1H, NH); $^{31}P$ (acetone-$d_6$) δ 150.2; FABMS m/z 885 ($MH\text{-}Et_3N^+$), 784 ($MH^+$), 303 (DMT); $^1H$ NMR (lower $R_f$ diastereomer, acetone-$d_6$) 1.12 (d, 6H, J = 6.8 Hz, $2CMe_2$), 1.17 (d, 6H, J = 6.8 Hz, $2CMe_2$), 1.76 (s, 3H, 5-$CH_3$), 2.01-2.06 (m, 1H, H of 5'-$CH_2CN$), 2.34 (dd, 1H, J = 17.0 Hz, H of 5'$CH_2CN$), 2.48-2.59 (m, 2H, H2'a, H2'b), 2.76 (t, 2H, J = 6.0 Hz, $CH_2CN$), 3.60-3.68 (m, 2H, $POCH_2$), 3.74-3.80 (m, 1H, $NCHMe_2$), 3.78, 3.79 (2s, 6H, $2OCH_3$), 3.85-3.91 (m, 1H, $NCHMe_2$), 4.01 (m, 1H, H5'), 4.20 (t, 1H, J = 4.3 Hz, H4'), 4.68 (m, 1H, H3'), 6.26 (t, 1H, J = 7.0 Hz, H1'), 6.88-6.92 (m, 4H, DMT), 7.23-7.57 (m, 9H, DMT), 7.60 (d, 1H, J = 1.2 Hz, H6), 10.07 (s, 1H, NH); $^{31}P$ (acetone-$d_6$) δ 150.4.

**5'(*R*)-*C*-Acetoxypropyl-5'-*O*-(4,4'-dimethoxytrityl)thymidine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) 30.** The procedure as described for **21** gave 221 mg (78.8%) of **30** as a colorless foam from 214 mg (0.33 mmol) of **28**, the higher $R_f$ diastereomer: $^1H$ NMR (acetone-$d_6$) δ 1.23 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.24 (d, 6H, J = 6.7 Hz, $CMe_2$), 1.26-1.65 (m, 7H, $2CH_2$, 5-$CH_3$), 1.87 (s, 3H, $CH_3CO$), 2.23-2.36 (m, 1H, H2'a, 2'b), 2.79 (m, 2H, $CH_2CN$), 3.39 (m, 1H, H4'), 3.58-3.92 (m, 12H, $COOCH_2$, $POCH_2$, $2NCHMe_2$, $2OCH_3$), 4.05 (m, 1H, H5'), 4.95 (m, 1H, H3'), 6.21 (dd, 1H, J = 9.2 Hz, 5.5 Hz, H1'), 6.88-6.92 (m, 4H, DMT), 7.11 (d, 1H, J = 1.3 Hz, H6), 7.20-7.59 (m, 9H, DMT); $^{31}P$ (acetone-$d_6$) δ 148.79; the lower $R_f$ diastereomer: $^1H$ NMR (acetone-$d_6$) δ 1.19 (d, 6H, J = 7.8 Hz, $CMe_2$), 1.21 (d, 6H, J = 7.7 Hz, $CMe_2$), 1.36-1.63 (m, 7H, $2CH_2$, 5-$CH_3$), 1.87 (s, 3H, $CH_3CO$), 2.23-2.29 (m, 1H, H2'a), 2.39-2.45 (m, 1H, H2'b), 2.80 (t, 2H, J = 5.3 Hz, $CH_2CN$), 3.39 (m, 1H, H4'), 3.62-3.97 (m, 6H, $COOCH_2$, $POCH_2$, $2NCHMe_2$), 3.776, 3.781 (2s, 6H, $2OCH_3$), 3.99 (m, 1H, H5'), 4.88 (m, 1H, H3'), 6.21 (dd, 1H, J = 8.8 Hz, 5.9 Hz, H1'), 6.88-6.92 (m, 4H, DMT), 7.11 (d, 1H, J = 1.4 Hz, H6), 7.19-7.59 (m, 9H, DMT); $^{31}P$ (acetone-$d_6$) δ 149.37.

**5'(*S*)-*C*-Allyl-3',5'-O-di(t-butyl)silylthymidine 31**. To a solution of 5'(*S*)-*C*-allylthymidine[14] (34 mg, 0.12 mmol) and pyridine (anhydrous, 0.1 mL) in DMF (anhydrous, 1 mL) at 0 °C was added di(t-butyl)silyl ditriflate (53 μL, 0.144 mmol). The resulting mixture was stirred at room temperature for 20 h, diluted with ethyl acetate, washed with brine six times, dried ($Na_2SO_4$), and concentrated. Chromatography on silica (EtOAc-hexanes 1:2) gave 42 mg of **31** as a white solid; $^1H$ NMR ($CDCl_3$) δ 1.02 (s, 9H, t-Butyl), 1.06 (s, 9H, t-butyl), 1.95 (d, 3H, J = 1.4 Hz, 5-$CH_3$), 2.30-2.49 (m, 4H, H2'a, H2'b, $CH_2C$=C), 3.91 (dd, 1H, J = 6.3 Hz, 9.3 Hz, H4'), 4.42 (dd, 1H, J = 9.0 Hz, 18 Hz, H5'), 4.49-4.56 (m, 1H, H3'), 5.11-5.21 (m, 2H, =$CH_2$), 5.90-6.04 (m, 1H, -CH=C), 6.18 (dd, 1H, J = 5.3 Hz, 6.6 Hz, H1'), 7.01 (d, 1H, J = 1.4 Hz, H6), 8.56 (s, 1H, NH); Anal. Calcd. for $C_{21}H_{34}N_2O_5Si$: C, 59.68; H, 8.11; N, 6.63. Found: C, 59.89; H, 8.11; N, 6.35.

**5'(*R*)-*C*-Allyl-3',5'-*O*-di(t-butyl)silylthymidine 32**. The same procedure as that for **31** gave 37 mg of **32** as a white solid from 5'(*R*)-*C*-allylthymidine[14] (32 mg, 0.11 mmol). $^1H$ NMR ($CDCl_3$) δ 1.00 (s, 9H, t-Butyl), 1.06 (s, 9H, t-butyl), 1.95 (d, 3H, J = 1.4 Hz, 5-$CH_3$), 2.24-2.56 (m, 4H, H2'a, H2'b, $CH_2C$=C), 3.31 (t, 1H, J = 9.0 Hz, H4'), 4.07-4.14 (m, 1H, H3'), 4.23 (dd, 1H, J = 9.0 Hz, 18 Hz, H5'), 5.09-5.18 (m, 2H, =$CH_2$), 5.87-6.01 (m, 1H, -CH=C), 6.20 (dd, 1H, J = 4.1 Hz, 7.2 Hz, H1'), 7.01 (d, 1H, J = 1.4 Hz, H6), 9.30 (s, 1H, NH); Anal. Calcd. for $C_{21}H_{34}N_2O_5Si$: C, 59.68; H, 8.11; N, 6.63. Found: C, 59.63; H, 7.72; N 6.50.



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