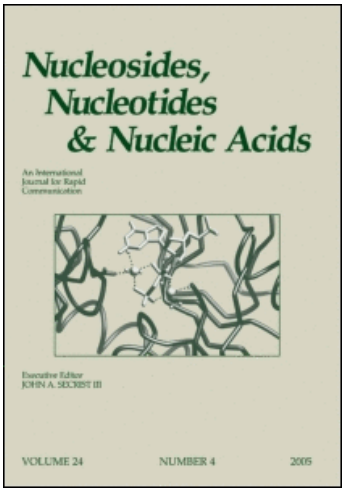

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Synthesis of 2'-Substituted Sulfide-Linked Dinucleotides

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SYNTHESIS OF 2'-SUBSTITUTED SULFIDE-LINKED DINUCLEOTIDES

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Abstract: 3'-Deoxy-3'-(2-mesyloxyethyl)ribofuranosylthymine derivative **3**, and its 2'-methoxy (**16**) and 2'-deoxy (**38**) analogs were condensed with 5'-deoxy-5'-thiothymidine **4** and **17** or 2'-O-methyl-5'-deoxy-5'-thiouridine **34** and **37** to provide, after standard functional group transformations, thymidine-thymidine and uridine-thymidine dimers **9**, **21**, **43** and **47**. Oxidation of model sulfide dimer **48** with oxone gave sulfone **49**. It was not stable to 27% ammonia.

INTRODUCTION

In response to the instability of DNA and RNA oligomers in vivo as well as problems regarding cellular uptake, a great deal of recent work has been devoted to the development of nucleic acid analogues for use as specific inhibitors of gene expression (the antisense strategy)[1]. In addition to oligonucleotides bearing modified phosphodiester groups, a number of antisense systems in which the phosphate group is replaced altogether have been described[2]. Apart from evaluating the suitability of antisense systems as potential therapeutics, the study of their DNA and RNA hybridization properties addresses the fundamental question of the role played by the backbone (sugar-phosphate units in natural strands) in the formation and stabilization of nucleic acid helices.

Our research in the antisense field has focused on oligonucleotide analogues in which phosphodiester groups are replaced by 3'-ethyl sulfide linkages[3]. We chose this modification as it is stable and nonhydrolyzable, and because examination of models

suggested that binding to complementary oligonucleotide targets would not be disrupted. In work previously carried out in our laboratory, two dinucleosides **9**[3d] and **9a**[3c] were synthesized and incorporated into a DNA dodecamer (GCG(TX)3GCT), respectively. DNA strands containing modified dimer **9** exhibited cooperative binding to both fully natural DNA and RNA, while DNA strands containing modified dimer **9** showed binding only to its complementary RNA, but not DNA. This selectivity of the latter, as reported in a preliminary communication[3d], was also confirmed by native gel analysis.

Here, we describe the preparation of four related mixed ribo-deoxyribo dinucleoside analogs **9**, **21**, **43** and **47** in which the two furanoses carry a hydroxy or methoxy group at the 2'-position. Our interest in this class of compounds was stimulated by the prospect that 2'-modification oligomers might have improving biochemical properties such as enhanced binding affinity[4].

The syntheses of these dinucleotide analogues, as well as their incorporation into oligomers, and the binding properties of the latter have been recently summarized[3e].

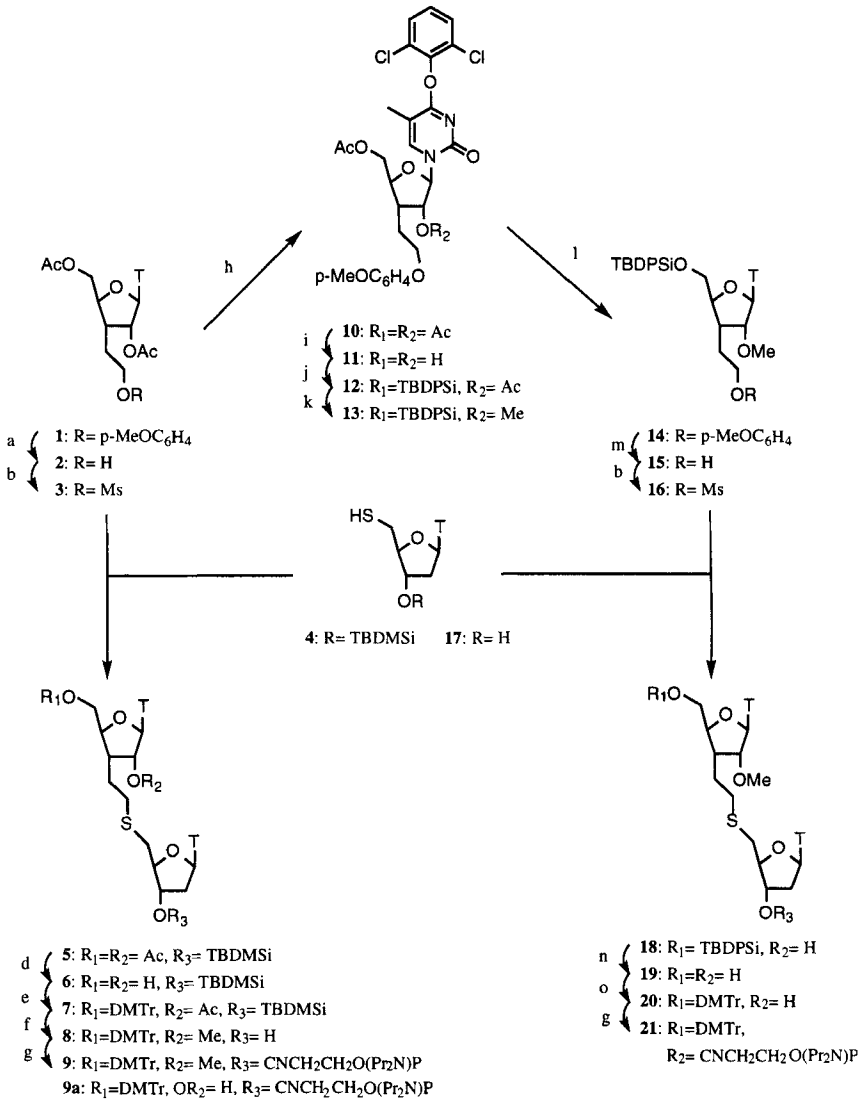
RESULTS AND DISCUSSION

Synthesis of hydroxy sulfide dimer 9.

Phosphoramidite **9** was synthesized from the 2-hydroxyethylthymine derivative **1**[3b,5] as outlined. The oxidative removal of the *p*-methoxyphenyl group was effected by treatment with ceric ammonium nitrate (CAN). Mesylation of the resulting alcohol **2** gave the 5'-end of the dimer in 74% yield. The coupling of mesylate **3** and thionucleoside **4**[3b] was carried out in deoxygenated *N,N*-dimethylformamide (DMF) employing cesium carbonate (84% yield) to avoid the otherwise rapid oxidation of the thiol to the disulfide under basic conditions. The deacetylation of **5** by ammonia gave diol **6** cleanly in 97% yield. The 5'-hydroxyl function was then selectively protected[6] by a dimethoxytrityl (DMTr) group, and the 2'-O-acetate group required during the subsequent solid-phase synthesis was immediately reintroductioned with acetic anhydride to yield **7** (76% yield). The 3'-silyl ether was cleaved with tetra-*n*-butylammonium fluoride (94%), and the resulting alcohol **8** was treated with 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite under standard conditions[7,8] to yield (68%) the appropriately activated dimer **9** for incorporation into DNA.

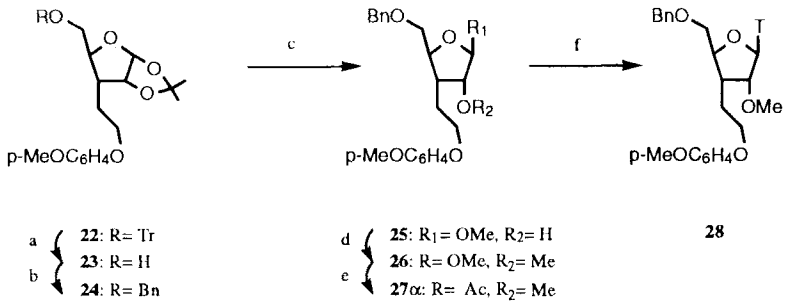
Synthesis of methoxy sulfide dimer 21.

Phosphoramidite **21** was also synthesized starting from thymidine derivative **1.** Because of competing N-methylation of the base, the base was protected using the method described by Nyilas and Chattopadhyaya[9] to produce **10** in 85% yield.



Synthesis of phosphoramidites **9** and **21**. a) CAN/H_2O/MeCN; b) MsCl/Et_3N/CH_2Cl_2; c) $CsCO_3$/DMF; d) NH_3/MeOH; e) DMTrCl/Et_3N/pyridine, then Ac_2O; f) TBAF/THF/Et_3N; g) *i*-$Pr_2NP(Cl)OCH_2CH_2CN$/Et_3N/CH_2Cl_2; h) 2,4,6-$(CH_3)_3C_6H_2SO_2Cl$/DMAP/Et_3N/CH_2Cl_2, then 2,6-Cl_2C_6HOH/DABCO; i) NaOH/MeOH/H_2O; j) TBDPSiCl/DMF/imidazole; k) NaH/MeI/DMF; l) 4-nitrobenzaldoxime/TMG/CH_3CN/H_2O; m) CAN/MeOH/MeCN; n) TBAF/THF; o) DMTrCl/Et_3N/pyridine.

Scheme 1



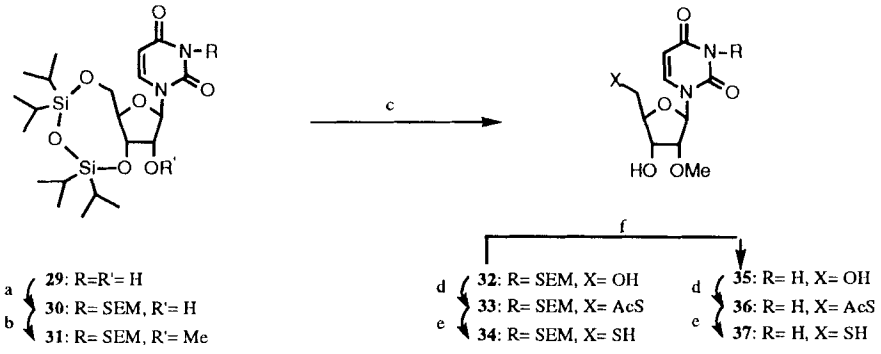
Synthesis of nucleoside **28**. a) Cl_3CCOOH/CH_2Cl_2; b) $NaH/Bu_4NI/BnBr/THF$; c) H /MeOH; d) NaH/MeI/DMF; e) $Me_2BBr/ClCH_2CH_2Cl$, then CH_3COOH/Et_3N; f)$(TMSi)_2$Thymine/$ClCH_2CH_2Cl/SnCl_4$.

Scheme 2

Deacetylation of **10** was carried out with 50% aqueous methanolic sodium hydroxide. The 5'-hydroxyl group of **11** was then transformed to its *t*-butyldiphenyl silyl ether, and the resulting diol monoether **12** was methylated using methyl iodide and sodium hydride in DMF. The deblocking of the base of **13** was effected with 4-nitrobenzaldoxime and tetramethylguanidine (TMG), and of the 2"-end of **14** with ceric ammonium nitrate (CAN). Transformation of **15** to **16** was accomplished as described for **2** to **3**. The methoxy sulfide dimer **18** was prepared by the coupling of mesylate **16** and thionucleoside **17** in DMF using cesium carbonate as base. Desilylation, dimethoxytritylation and phosphoramidite formation were carried out as described for the formation of **9**. The activated dimer **21** was obtained in a mixture of diastereomers at phosphorus after purification by chromatography.

We also investigated the route starting from the readily available xylose derived **22**[5] (Scheme 2). Its detritylation under acidic conditions provided alcohol **23**, which was benzylated. The resulting benzyl ether **24** was subjected to acidic methanolysis, followed by methylation with methyl iodide and sodium hydride. The desired 2'-O-methyl derivative **26** was obtained in 66% yield. Acetolysis of **26** with $AcOH/Ac_2O/H^+$ did not provide the desired acetate. Acetal **26** was therefore treated with dimethylboron bromide[10] in 1,2-dichloroethane at -78°C, and the resulting bromo derivative was immediately reacted with acetic acid and triethylamine. The acetate **27** was obtained as an 6:1 mixture (by ^{1}H-NMR) of α and β-anomers.

The α-anomer was obtained in a pure state by chromatography, and could be converted in 46% yield to **28**, obtained exclusively as the β-anomer when using disilylated thymine and



(a) $ClCH_2O(CH_2)_2SiMe_3$/diisopropyl ethylamine; (b) CH_3I/Ag_2O; (c) TBAF/THF; (d) AcSH/DIAD/Ph_3P/THF; (e) MeOH/NH_3; (f) TBAF/THF, reflux.

Scheme 3

redistilled $SnCl_4$ in 1,2-dichloroethane. The β-configuration of **28** was confirmed by ^{1}H-NMR, COSY and nOe experiments.

Synthesis of 2'-O-methyl uridines 34 and 37.

Direct methylation using published procedures[11] did not provide the desired 2'-*O*-methyl uridine in adequate purity and yield. Therefore we protected the 3' and 5'-OH groups by forming the 3'-*O*, 5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine **29**[12]. Methylation of **29** with methyl iodide and 2-*tert*-butylimino-2-diethyl-amino-1,3-dimethylperhydro-1,3,2-diazaphosphorine (BDDDP)[13] in acetonitrile provided the undesired *N*-methyluridine. Its structure was confirmed by ^{1}H-NMR, which showed a peak at 3.30 ppm rather than the expected 3.6 ppm, characteristic for the methoxy group.

To prevent this methylation, the imide group was protected as its (trimethylsilyl) ethoxymethyl (**SEM**) derivative **30** with 1.05 eq 2(trimethylsilyl)ethoxy methyl chloride, originally developed to protect hydroxy groups[14]. Methylation of the 2'-OH group of **30** by heating in iodomethane containing silver dioxide afforded 2'-*O*-methylated uridine **31** quantitatively. The 3', 5'-*O*-tetraisopropyldisiloxane-1,3-diyl group of **31** was removed with tetrabutylammonium fluoride (TBAF) in THF at room temperature, giving diol **32** in 76 % yield.

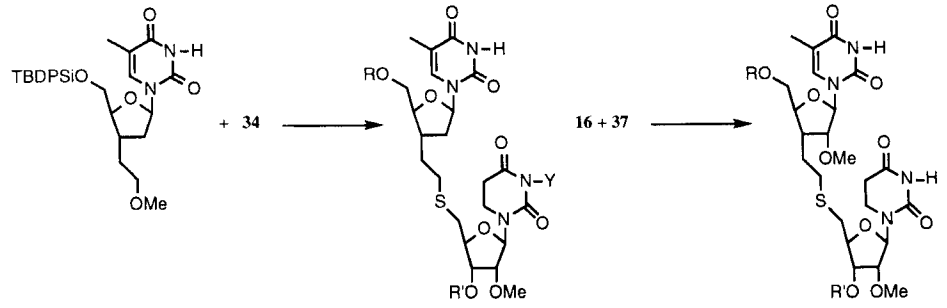
The SEM group was removed with TBAF in refluxing THF and provided 2'-*O*-methyluridine **35** in 95 % yield. Alternatively, both desilylations could be carried out simultaneously, providing **35** directly from **31**. Alcohol **32** was transformed to thiol acetate

33 by a Mitsunobu reaction[15] in 62 % yield. Deacetylation with ammonia in methanol then gave thiol **34** in 98 % yield. It was used as the "3'-end unit" monomer for the synthesis of thymidine / 2'-*O*-methyluridine dinucleoside **43**. Thiol **37** was synthesized by using the above procedures starting from 2'-*O*-methyluridine **35**.

Thiols **34** and **37** were very easily oxidized to the corresponding disufide, and their formation was difficult to avoid. They could be reduced to the thiols with triphenylphosphine in dioxane and water[16].

Synthesis of Dimers 43 and 47.

Most of the procedures used for the synthesis of dimer **43** were similar to the one described in the following scheme except the following modifications which took advantage of the properties of the SEM protecting group. The 5'-*O*-TBDMS group of dimer **39** was removed by TBAF in THF at 0 °C giving **40** quantitatively without touching the 3-*N*-SEM group. Only one of the silyl protecting groups was removed in order to maintain higher solubility. After transformation of alcohol **40** to its dimethoxytrityl ether **41**, the 3-*N*-SEM protecting group of dimer **41** was removed with TBAF in THF containing 10 % of triethylamine by refluxing for 20 hours giving dimer **42** in 98 % yield.

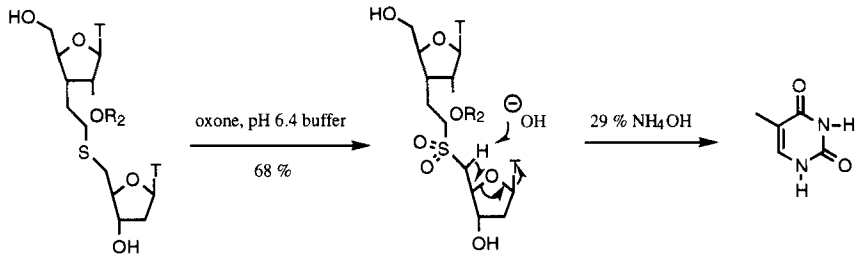


b, c, d, e: **39**: R= TBDMSi, R'= H, Y= SEM; **40**: R=R'= H, Y= SEM; **41**: R=DMTr, R'= H, Y= SEM; **42**: R=DMTr, R'=Y= H; **43**: R=DMTr, R'= $CNCH_2CH_2O(Pr_2N)P$, Y= H

b, c, e: **44**: R= TBDMSi, R'= H; **45**: R=R'= H; **46**: R=DMTr, R'= H; **47**: R=DMTr, R'= $CNCH_2CH_2O(Pr_2N)P$

a) $CsCO_3$/DMF; (b) TBAF/THF; (c) 4,4'-Dimethoxytrityl chloride/pyridine/DMAP; (d) TBAF/THF/reflux; (e) 2-Cyanoethyl-*N,N'*-diisopropylchlorophosphoramidite/ CH_2Cl_2/Et_3N.

Scheme 4



48 49

Scheme 5

Standard phosphitylaton then provided phosphoramidite **43**. Its ^{31}P-NMR spectrum showed the anticipated signals in CD_3OD at 150.59 and 150.95 ppm.

3'-Deoxy-3'-(2-mesyloxyethyl)thymine derivative **16** and thiol **37** were coupled with cesium carbonate in DMF as described. Dimer **44** was then desilylated. Dimethoxytritylation and phosphitylation gave the desired dimer **47**.

Sulfone 49.

Oxidation of sulfide **48**[3c] with oxone in a pH 6.4 buffer gave sulfone **49** in excellent yield. Unfortunately, it was not stable to 29% ammonia at room temperature for one hour, and the only product isolated and identified from the latter reaction was thymine, probably formed as shown in scheme 5.

Incorporation into oligomers, and binding properties of the sulfide dimers are described in a forthcoming paper[17].

EXPERIMENTAL PROCEDURES

General methods

^{1}H- and ^{13}C-NMR were recorded on Varian XL200 and XL300 spectrometers. Chemical shifts are given in ppm (d) with respect to tetramethylsilane for ^{1}H, and respect to $CDCl_3$ (77.00 ppm), CD_3OD (49.00 ppm), or CD_3COCD_3 (30.00 ppm) for ^{13}C. ^{31}P-NMR spectra were obtained on a Varian XL200 at 81.0 MHz and are given relative to 85% phosphoric acid as external reference.

Alcohols 2 and 15

Ceric ammonium nitrate (11.85 g, 21.6 mmole) was added to a solution of **1** (4.82 g, 10.1 mmole) in acetonitrile-water (1 : 1, 56 ml) at 0°C. The reaction was stirred at 0°C

for 30 min and diluted with brine (116 ml). The mixture was extracted with ethyl acetate (3 x 200 ml). The organic extracts were washed with sodium sulfite (10% w/v, until the aqueous layer remained colorless), sodium bicarbonate (5% w/v, 100 ml), and dried (Na_2SO_4). Removal of the solvent yielded a yellow foam which was chromatographyed over silica gel (20 : 1 CH_2Cl_2 / MeOH, v/v) and afforded the alcohol **2** (2.83g, 76%), m.p. 126-127°C (EtOH / H_2O). ^1H-, ^{13}C-NMR and MS data were consistent with the structure.

Alcohol **15** (348 mg, 72%) was obtained from **14** as described above except that acetonitrile-methanol was used as solvent: m.p. 68-70°C (EtOH / H_2O); ^1H- and ^{13}C-NMR were consistent with the structure; HRMS (CI-NH_3) m/e calcd. for $C_{29}H_{38}N_2O_6Si$ + H^+: 539.25775, found: 539.26490.

Mesylates 3 and 16

Methanesulfonyl chloride (0.48 ml, 6.20 mmole) was added to a stirred solution of alcohol **2** (1.00 g, 2.70 mmole) and dry triethylamine (0.68 ml, 4.88 mmole) in dry dichloromethane (19 ml) at room temperature under nitrogen. After 1 h, the reaction was diluted with dichloromethane (20 ml) and washed with hydrochloric acid (5% w/v, 7 ml), saturated aqueous sodium bicarbonate (7 ml), and brine (5% w/v, 7 ml). The organic layer was dried (Na_2SO_4), and the solvent was removed yielding a yellow foam. Chromatography over silica gel (20 : 1 CH_2Cl_2 / MeOH, v/v) afforded the mesylate **3** as a white foam (1.17 g, 97%): ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 12.51 ppm (5-Me), 20.57 (2 x CO**Me**), 24.25 (C1"), 37.23 (SO_2Me), 38.34 (C3'), 62.94 (C5'), 67.98 (C2"), 77.00 (C2'), 81.784 (C4'), 91.77 (C1'), 110.72 (C5), 136.39 (C6), 150.31 (C2), 164.28 (C4), 160.99, 170.62 (**C**OMe); MS (FAB-NBA) m/e 449 ([MH^+], 46 %), 389 ([MH^+ - AcOH], 5.7), 323 ([MH^+ - ThyH], 100); HRMS (FAB-glycerol) m/e calcd. for $C_{17}H_{24}N_2O_{10}$ + H^+: 449.12300, found: 449.12316.

Mesylate **16** (406 mg, 87%) was obtained from **15** as described above: ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 12.01 ppm (5-Me), 19.37 (**C**Me_3), 23.84 (C1"), 26.99 (C**Me**$_3$), 37.26 (C3'), 58.00 (2'-OMe), 60.24 (C5'), 62.15 (C2"), 67.70 (SO_2CH_3), 84.46 (C2'), 84.91 (C4'), 88.59 (C1'), 110.52 (C5), 127.72-135.33 (C6 and Ph), 150.33 (C2), 164.14 (C4); HRMS (FAB - glycerol) m/e calcd. for $C_{30}H_{40}N_2O_8SSi$ + H^+: 617.23530, found: 617.23527.

Dimers 5 and 18

Cesium carbonate (549 mg, 1.68 mmol) was suspended in dry, deoxygenated DMF (9 ml) and a deoxygenated solution (saturated with N_2 over 20 min.) of **3** (505 mg, 1.17 mmol) and **4** (479 mg, 1.29 mmol) in DMF (14 ml) was added into it under nitrogen.

The reaction mixture was stirred for 1 h. The solvent was evaporated, and the residue extracted with dichloromethane (2 x 270 ml) and washed with aqueous sodium bicarbonate (5% w/v, 225 ml) and water (225 ml). Chromatography of the crude material over silica gel (1 : 2.5 EtOAc / hexanes, v/v) afforded the dimer **5** (711 mg, 84%), m.p. 83-86°C (EtOH / H_2O): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.092 (s, 6H, $SiMe_2$), 0.90 (s, 9H, CMe_3), 1.60-1.80 (m, 2H, ^{5}H1"),1.92 and 1.93 (two s, 6H, 2 x 5-Me), 2.10 and 2.15 (two s, 6H, 2 x COMe), 2.18-2.26 (m, 2H, ^{3}H2'), 2.41-2.51 (m, 1H, ^{5}H3'), 2.51-2.70 (m, 2H, ^{5}H2"), 2.75 (A of ABX, 1H, ^{3}H5'$_A$), 2.78 (B of ABX, 1H, ^{3}H5'$_B$), 3.94 (q, 1H, ^{5}H4'), 4.10 (dt, 1H, ^{3}H4'), 4.28-4.37 (m, 3H, ^{3}H3' and ^{5}H5'$_{AB}$), 5.45 (d, 1H, ^{5}H2'), 6.21 (s, 1H, ^{5}H1'), 6.21 (t, 1H, ^{3}H1'), 7.30 and 7.56 (two s, 2H, H6), 9.01 (br s, 2H, 2 x NH), $J_{3H1'-3H2'}$ = 6.6 Hz, $J_{5H2'-5H3'}$ = 6.0, $J_{5H3'-5H4'}$ = 7.5, $J_{3H3'-3H4'}$ = 4.5, $J_{3H4'-3H5'A}$ = 5..7 $J_{3H4'-3H5'B}$ = 4.9, $^2J_{3H5'A-3H5'B}$ = -13.8; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.11 (2 x 5-Me), 17.12 (**C**Me_3), 20.22 and 20.32 (2 x **COMe**), 24.31 (^{5}C5'), 25.21 (Si**Me$_2$** and **CMe$_3$**), 30.50 (^{5}C2"), 33.49 (^{3}C5') 39.87 (^{5}C2'), 40.16 (^{3}C3'), 62.88 (^{5}C5'), 73.01 (^{3}C3'), 76.67 (^{5}C2'), 81.69, 84.54, 85.18 (^{3}H1' and 2 x H4'), 91.27 (^{5}C1'), 110.25, 110.70 (2 x C5), 135.35, 135.62 (2 x C6), 149.82, 150.10 (2 x C2), 163.79, 163.87 (2 x C4), 169.21, 169.94 (**C**OMe); MS (FAB - NBA) m/e 725 ([MH$^+$], 7.5 %), 599 ([MH$^+$ - ThyH], 52), 473 ([MH$^+$ - 2 x ThyH], 12), 399 ([MH$^+$ - 2 x ThyH - MeCOOMe], 33), 341 (57), 295 (17), 213 (100).

Dimer **18** (86 mg, 84%) was prepared by the coupling of **16** and **17** as described above, m.p. 116-118°C (AcOEt / hexanes): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.07 (s, 9H, CMe_3), 1.44 (s, 3H, ^{3}5-Me), 1.74-1.87 (m, 2H, ^{5}H1"), 1.88 (s, 3H, ^{5}5-Me), 2.18-2.41 (m, 3H, ^{3}H2' and ^{5}H3'), 2.44-2.62 (m, 2H, ^{5}H2"), 2.79 (d, 2H, ^{3}H5'), 3.55 (s, 3H, ^{5}2'-OMe), 3.73 (dd, 1H, ^{5}H4'), 3.85 (d, 1H, ^{3}H4'), 3.97-4.02 (m, 2H, ^{5}H5'$_A$ and ^{5}H2'), 4.18 (d, 1H, ^{5}H5'$_B$), 4.35 (dt, 1H, ^{3}H3'), 5.85 (s, 1H, ^{5}H1'), 6.21 (t, 1H, ^{3}H1'), 7.25-7.63 (m, 12H, Ph and 2 x H6), 9.23 and 9.43 (br s, 1H, 2 x NH), $J_{5H1'-5H2'}$ = 0, $J_{5H2'-5H3'}$ = 5.0, $J_{5H3'-5H4'}$ = 2.5, $J_{3H1'-3H2'}$ = 6.6, $J_{3H3'-3H4'}$ = 5.0, $J_{3H2"-3H3'}$ = 7.2; ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.94 and 12.54 ppm (2 x 5-Me), 19.41 (**C**Me_3), 23.95 (^{5}C1"), 27.03 (**CMe$_3$**), 31.38 (^{5}C2"), 34.42 (^{3}C5'), 39.80 (^{5}C3'), 39.93 (^{3}C2'), 58.02 (^{5}2'-OMe), 62.49 (^{5}C5'), 73.07 (^{3}C3'), 84.61, 84.68, 85.06 (^{5}C2', ^{3}C1' and 2 x C4'), 88.66 (^{5}C1'), 110.32, 111.05 (2 x C5), 127.72-135.61 (C6 and Ph), 150.49 (2 x C2), 164.01, 164.34 (2 x C4); m/e calcd. for $C_{39}H_{50}N_4O_9SSi$ + H$^+$: 779.31461, found: 779.31438.

Diol 6

Dimer **5** (518 mg, 0.72 mmol) was suspended in dry methanol (10 ml) and cooled to 0°C. The mixture was then saturated with ammonia gas and allowed to warm to room temperature. After 11 h the resulting homogeneous solution was evaporated yielding a

white foam. Chromatography over silica gel (20:1 CH_2Cl_2 / MeOH, v/v) afforded the diol **6** (442 mg, 97% yield), m.p. 108-110°C (AcOEt / hexanes): ^{1}H-, ^{13}C-NMR and MS data were consistent with the structure.

Dimethoxytrityl Ethers 7 and 20

To diol **6** (220 mg, 0.34 mmole) in dry pyridine (3.4 ml) were added dimethoxytrityl chloride (330 mg, 0.97 mmole) and triethylamine (0.18 ml, 1.29 mmole) in three portions over an 8 h period. After the reaction was completed, acetic anhydride (2.00 ml, 21.2 mmole) was added and the solution was kept overnight at room temperature under nitrogen. Saturated sodium bicarbonate (30 ml) was added and the resulting solution was extracted with dichloromethane (2 x 30 ml). The combined organic layers were washed with water (30 ml), dried (Na_2SO_4) and evaporated. Chromatography over the silica gel (100 : 5 : 1 CH_2Cl_2 / MeOH / Et_3N, v/v) afforded the dimethoxytrityl ether **7** (0.246 mg, 76%). ^{1}H-, ^{13}C-NMR and MS data were consistent with the structure.

Dimethoxytrityl ether **20** (168 mg, 83%) was prepared from diol **19** using the same method described above for the tritylation of diol **6**: m.p. 78-81°C (EtOH / H_2O); ^{13}C-NMR (75.4 MHz, acetone) δ 11.78, 11.92 ppm (2 x 5-Me), 26.18 (^{5}C1"), 30.69 (^{5}C2"), 34.84 (^{3}C5'), 39.81 (^{5}C3'), 40.97 (^{3}C2'), 46.34 (Ph$_3$**C**), 55.49 (PhO**Me**), 58.45 (^{5}2'-O**Me**), 62.60 (^{5}C5'), 73.91 (^{3}C3'), 84.65, 85.15, 86.31 (^{5}C2', ^{3}C1' and 2 x C4'), 87.15 (^{5}C1'), 109.99, 110.90 (2 x C5), 127.20-145.56 (Ph and 2 x C6), 151.05, 151.21 (2 x C2), 159.67, 164.36 (2 x C4); HRMS (FAB-glycerol) m/e calcd. for $C_{44}H_{50}N_4O_{11}S$ + H^+: 843.32751, found: 843.32776.

Dimethoxytritylated alcohol 8 and Diol 19

A solution of tetra-*n*-butylammonium fluoride in tetrahydrofuran (1 M, 0.647 mL, 0.647 mmol) was added to a stirred solution of dimethoxytrityl ether **7** (255 mg, 0.259 mmol) and triethylamine (0.15 ml,1.17 mmole) in dry tetrahydrofuran (7 mL). After 1 h the solution was evaporated and the resulting foam was chromatographyed over silica gel (100 : 5 : 1 CH_2Cl_2 / MeOH / Et_3N, v/v) to give the dimethoxytritylated alcohol **8** (212 mg, 94%), m.p. 92-95°C (EtOH / H_2O): ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 10.63 (2 x 5-Me), 21.20 (CO**Me**), 25.10 (^{5}C1"), 31.10 (^{5}C2"), 34.76 (^{3}C5'), 40.23 (^{3}C2'), 40.32 (^{5}C3'), 46.31 (Ph$_3$**C**), 55.61 (2 x O**Me**), 62.16 (^{5}C5'), 73.25 (^{3}C3'), 77.70 (^{5}C2), 83.91, 85.30 (2 x H4'), 90.31 (2 x H1'), 111.14 and 111.38 (2 x C5), 113.53 -144.37 (Ph and C6), 150.60, 150.74 (2 x C2), 158.88 (Ph), 164.29 , 164.49 (2 x C4), 170.05 (COMe); MS (FAB - NBA) m/e 871 ([MH$^+$], 59%), 745 ([MH$^+$ - ThyH], 28), 551 ([MH$^+$ - DMTrOH], 87); HRMS (FAB - Glycerol) m/e Calcd. for $C_{45}H_{50}N_4S$ + H^+: 871.32242, found: 871.32203.

Diol **19** (45 mg, 81%) was obtained from **18** as described above, but without using Et_3N: m.p. 167-169°C; ^1H-and ^{13}C-NMR were consistent with the structure; HRMS (FAB-glycerol) m/e calcd. for $C_{23}H_{32}N_4O_9S + H^+$: 541.19683, found: 541.19668.

Phosphoramidites 9 and 21

2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.137 mL, 0.62 mmol) was slowly added to a stirred solution of tritylated alcohol **8** (265 mg, 0.31 mmol) in dry dichloromethane (4 mL) containing triethylamine (0.168 mL, 1.20 mmol). After 6 h of stirring at room temperature under nitrogen, the solution was diluted with ethyl acetate (87 mL) and washed with brine (4 x 173 mL). The organic layer was then dried (Na_2SO_4), and evaporated yielding a pale yellow foam. Chromatography over silica gel (100 : 5 : 1 CH_2Cl_2 / MeOH / Et_3N, v/v) afforded the phosphoramidite **9** as a white foam (234 mg, 72%), which was used as such in the subsequent solid-phase syntheses: ^{31}P-NMR (81.0 MHz, acetone) δ 150.91 and 151.03 ppm; MS (FAB - NBA) m/e 1072 ([MH^+], 10%), 946 ([MH^+ - ThyH], 8), 853 ([MH^+ - *i*$Pr_2NP(OH)OCH_2CH_2CN$], 5), 768 ([MH^+ - DMTrH], 9), 551 (16), 457 (100).

Amidite **21** (195 mg, 95%) was prepared from dimethoxytrityl ether **20** as described above: ^{31}P-NMR (81.0 MHz, acetone) d 150.85 and 151.03 ppm; MS (FAB - NBA) m/e 1043 ([MH^+], 0.5%), 399 (69.7), 303 ([$DMTr^+$], 100), 219 ([*i*$Pr_2NH^+P(OH)OCH_2CH_2CN$], 25.2).

O^4-(2,6-Dichlorophenyl]-diacetate 10

To compound **1** (1.40g, 2.94 mmole) in CH_2Cl_2 (28 ml) and NEt_3 (16 ml, 114.80 mmole), was added 2-mesitylenesulfonylchloride (2.47 g, 11.29 mmole) and DMF (192 mg, 1.56 mmole). After 30 min, 2,6-dichlorophenol (2.42 g, 14.8 mmole) and 1,4-diazabicyclo[2,2,2]octane (65 mg, 0.58 mmole) were added and the mixture stirred for another 1 hr. The solution was washed with saturated sodium bicarbonate and dried over sodium sulfate. The solvents were evaporated and the residue was purified on a silica gel column (3 : 1 AcOEt / hexanes, v/v) to give **10** (1.54 g, 85%), m.p. 78-80°C (EtOH / H_2O); ^1H- and ^{13}C-NMR were consistent with the structure; HRMS (CI-NH_3) m/e calcd. for $C_{29}H_{30}N_2O_9Cl_2 + H^+$: 621.14067, found: 621.14073.

O^4-[2,6,-Dichlorophenyl] diol 11

Diacetate **10** (1,54 g, 2.48 mmole) was dissolved in cold (0°C) methanol (17 ml) and to this solution was added a 50% aqueous methanolic solution of sodium hydroxide (1 N, 3 ml). After 1 h of stirring at 0°C, the solution was diluted with water (15 ml) and extracted with dichloromethane (3 x 20 ml). The organic layer was dried over sodium

sulfate and the solvent was removed. The residue was chromatographyed (3 : 1 AcOEt / hexane, v/v) to give diol **11** (1.21 g, 91%), m.p. 196-197°C (CH_2Cl_2 / hexanes); ^1H-, ^{13}C-NMR and MS were consistent with the structure.

O^4-[2,6,-Dichlorophenyl]-5'-silyl ether 12

To a solution of diol **11** (1.21 g, 2.26 mmole) in freshly distilled DMF (4.2 ml) was added *tert*-butyldiphenylsilyl chloride (1.22 ml, 4.58 mmole) and imidazole (315 mg, 4.58 mmole). The solution was stirred for 6 h at room temperature and then poured into water. The product was extracted with dichloromethane (150 ml) and washed with water (100 ml). The combined organic extracts were then dried (Na_2SO_4), filtered and the solvent removed in *vacuo*. The residue was chromatographyed over silica gel (3 : 1 EtOAc / hexanes, v/v) to give silyl ether **12** (1.42 g, 81%): m.p. 88-90°C (EtOH / H_2O); ^1H-, ^{13}C-NMR and MS were consistent with the structure.

O^4-[2,6,-Dichlorophenyl]-2'-O-methyl ether 13

Sodium hydride (60% oil disp., 328 mg, 8.22 mmole) and methyl iodide (0.78 ml, 12.26 mmole) were successively added to a cooled (0°C) solution of alcohol **12** (1.41 g, 1.82 mmole) in freshly distilled DMF, and the reaction mixture was stirred for 1 h at 0°C. The remaining hydride was destroyed by the carefully addition of water and the resulting solution extracted with dichloromethane (3 x 60 ml). The combined organic extracts were dried (Na_2SO_4), filtered and the solvent removed in *vacuo* yielding a yellow syrup. Chromatography over silica gel (4 : 1 EtOAc / hexanes, v/v) afforded methyl ether **13** (1.40 g, 98%), m.p. 65-66°C (EtOH / H_2O): ^1H-, ^{13}C-NMR and MS were consistent with the structure.

2'-O-Methyl ether 14

O^4-[2,6,-Dichlorophenyl]-2'-O-methyl ether **13** (0.84 g, 1.06 mmole) was dissolved in methanol-acetonitrile (1 : 1, v/v, 9 ml) and to this solution 4-nitrobenzaldoxime (1.77 g, 10.60 mmole) and 1,1,3,3-tetramethylguanidine (1.34 ml, 10.6 mmole) were added at room temperature. After 10 h, the solvent was evaporated, and the residue dissolved in dichloromethane (30 ml) and washed with water (2 x 15 ml). The organic layer was dried (Na_2SO_4) and concentrated. The residue was purified on a silica gel column (3 : 1 EtOAc / hexanes, v/v) to give **14** (582 mg, 85%): m.p. 62-64°C (EtOH / H_2O); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 11.73 ppm (5-Me), 19.31 (**C**Me_3), 23.59 (C1"), 26.96 (C**Me**$_3$), 37.35 (C3'), 55.43 (PhOMe), 57.95 (2'-OMe), 62.11 (C5'), 66.18 (C2"), 84.93 (C2'), 85.35 (C4'), 88.65 (C1'), 110.15 (C5), 114.74-135.25 (Ph), 138.31 (C6), 150.74, 152.96 and 153.87 (C2 and Ph), 164.77(C4); HRMS (CI-NH_3) m/e calcd. for $C_{36}H_{44}N_2O_7Si + H^+$: 645.29961, found: 645.29981.

Alcohol 23

To trityl ether **22** (668 mg, 1.18 mmole) in dichloromethane (16 ml) was added dropwise a solution of trichloroacetic acid in dichloromethane (1:4 w/v, 5.6 ml). After 3 h of stirring at room temperature, the reaction mixture was diluted with chloroform (30 ml), washed with saturated aqueous sodium bicarbonate (60 ml) and water (60 ml). The organic phase was then dried (Na_2SO_4), filtered and evaporated. Chromatography of the crude product over silica gel (1:1 EtOAc / hexanes, v/v) afforded alcohol **23** (271 mg, 71%) as a colorless syrup: ^1H- and ^{13}C-NMR were consistent with the structure; HRMS (CI-NH_3) m/e calcd. for $C_{17}H_{24}O_6 + NH_4^+$: 343.19949, found: 343.19020.

2'-O-Methyl Ether 26, *via* 24 and 25

Sodium hydride (60% oil disp., 35 mg, 0.81 mmole) was added to a solution of alcohol **23** (260 mg, 0.80 mmole) in THF (1.5 ml) at room temperature, and the reaction mixture was stirred for 30 min. Then tetra-n-butyl ammonium iodide (30 mg, 0.08 mmole) and benzyl bromide (0.19 ml, 1.60 mmole) were added and the mixture was stirred for another 5 h. The reaction was quenched with water (5 ml) and extracted with dichloromethane (2 x 15 ml). The combined organic phases were washed with brine (5 ml), dried (Na_2SO_4), filtered and evaporated. The crude **24** was then dissolved in methanol (10 ml) and to this solution was added camphorsulfonic acid (562 mg, 2.42 mmole). The solution was refluxed for 15 min, cooled in ice and slowly added to aqueous sodium bicarbonate (5%, 30 ml). The mixture was stirred for 30 min and extracted with dichloromethane (2 x 100 ml). The combined organic layers were then dried (Na_2SO_4), filtered and evaporated, yielding **25** as a yellow syrup. To a cooled (0^oC) solution of alcohol **25** in DMF (3.3 ml) was added sodium hydride (60% oil disp., 40 mg, 1.01 mmole), and the reaction mixture was stirred for 45 min at 0^oC. Then methyl iodide (0.06 ml, 0.93 mmole) was added, and stirring continued for another 45 min at 0^oC. The excess sodium hydride was destroyed by slowly adding water, and the mixture extracted with ethyl EtOAc (2 x 40 ml). The combined organic layers were dried (Na_2SO_4), filtered and evaporated. Chromatography of the crude product over silica gel (1:4 AcOEt / hexanes, v/v) gave **26** as a colorless syrup (212 mg, 66% yield from **23**): ^{13}C-NMR (55.5 MHZ, $CDCl_3$) δ 25.41 (C1'), 40.51 (C3), 54.45 (1-OMe), 55.72 (PhOMe), 57.94 (2-OMe), 67.17 (C5), 73.25 (C2'), 73.51 (Ph**CH2**), 83.15 (C2), 85.06 (C4), 1005.46(C1), 114.63-153.76 (CPh); MS (CI-NH_3) m/e 420 ([M + NH_4^+], 6.8%), 402 ([M], 25.9), 371 ([MH^+ - C_3H_6O], 100), 157 (100).

Acetate 27

To a stirred solution of **26** (148 mg, 0.37 mmole) in 1,2-dichloroethane (4 ml) was added dimethylboron bromide (0.60 ml, 1.20 mmole) at -78°C under nitrogen. After 1.5

h, acetic acid (0.30 ml, 5.23 mmole) and triethylamine (1.00 ml, 6.75 mmole) were added, and the solution was warmed to 0°C and stirred for another 30 min. The reaction solution was diluted with CH_2Cl_2 (5 ml) and washed with saturated $NaHCO_3$. The aqueous solution was extracted with CH_2Cl_2 (2 x 5 ml). The combined organic layers were dried (Na_2SO_4), filtered and evaporated. The crude compound was chromatographyed over silica gel (1:3 hex / AcOEt) to give **27** (140 mg, 88%, a:b = 6:1 by NMR) as a colorless syrup. The α-isomer was obtained by carefully separating the mixture on a silica gel column (1:1 hex / AcOEt). For α-isomer: ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 21.38 (CO**Me**), 27.76 (C1'), 37.68 (C3), 55.71 (PhO**Me**), 59.15 (2-OMe), 67.38 (C5), 71.40 (C2'), 73.46 (Ph**CH2**), 83.06 (C2), 84.23 (C4), 95.61 C1), 114.61-153.75 (CPh), 170.25 (**C**OMe); HRMS (FAB-Glycerol) m/e calcd. for $C_{24}H_{30}O_7$ + H^+: 431.20698, Found 431.20709.

2-O-methyl thymidine analogue 28

To a cooled (0°C) solution of acetate **27** (116 mg, 0.27 mmole) and disilylated thymine (77 mg, 0.28 mmole) in 1,2-dichloroethane (1.6 ml), a solution of redistilled $SnCl_4$ (0.02ml, 0.19 mmole) in 1,2-dichloroethane was added with vigorous stirring. The reaction was finished after 16 h at room temperatures, and the solution was diluted with dichloromethane (5 ml) and washed with saturated $NaHCO_3$. The organic phase was separated, dried (Na_2SO_4) and evaporated. The crude product was purified over a silica gel column (3 : 1 AcOEt / hexanes, v/v) to give **28** (64 mg, 48%) as a white foam: ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.51 (s, 3H, 5-Me), 1.70 (m, 1H, H1"$_A$), 2.05 (m, 1H, H1"$_B$), 2.52 (h[7], 1H, H3'), 3.58 (s, 3H, 2'-OMe), 3.64 (m, 2H, H5'), 3.76 (s, 3H, PhOMe), 3.90-4.26 (m, 4H, H2", H2' and H4'), 4.46 (d, 2H, $PhCH_2$, J = 2.1 Hz), 5.91 (s, 1H, H1'), 6.78-7.35 (m, 9H, Ph), 7.88 (s, 1H, H6), 8.30 (s, 1H, NH); J_{H1-H2} = 0, J_{H2-H3} = 4.0; ^{13}C-NMR (55.5 MHz, $CDCl_3$) δ 11.71 ppm (5-Me), 23.60 (C1"), 36.78 (C3'), 55.03 (PhO**Me**), 57.28 (2'-OMe), 65.82 (C5'), 66.80 (C2"), 72.64 (Ph**CH2**), 83.26 (C2'), 84.75 (C4'), 87.31 (C1'), 109.70 (C5), 113.11-127.04 (Ph), 134.41 (C6) 150.90 (C2), 161.73 (C4); HRMS (FAB-Glycerol) m/e calcd. for $C_{27}H_{32}N_2O_7$ + H^+: 497.22878, found: 497.2290

3-*N*-(Trimethylsilyl)ethoxymethyl-3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)uridine 30.

To uridine **29** (486 mg, 1 mmol) in dry CH_2Cl_2 (5 mL) containing diisopropyl ethylamine (0.87 mL) was added 2-(trimethylsilyl)ethoxymethyl chloride (0.27 mL) at 0°C. The reaction left overnight at RT. The solvent was evaporated and the residue was chromatographed over silica gel (2:1 hexanes/ethyl acetate, v/v) to afford **30** as a white

foam (460 mg, 75 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.95 (t, 2H), 1.00-1.08 (m, 28H), 3.67 (t, 2H), 3.98 (m, 1H), 4.06-4.16 (m, 2H), 4.19 (m, 1H), 4.78 (dd, 1H), 5.36 (s, 2H), 5.71 (d, 1H), 5.78 (fine d, 1H), 7.90 (d, 1H), $J_{H1'-H2'}$ = 1.4 Hz, $J_{H2'-H3'}$ = 4.8, $J_{H3'-H4'}$ = 8.7, J_{H5-H6} = 8.2; ^{13}C NMR ($CDCl_3$) δ 12.14-13.05, 16.62-17.72, 17.72, 59.88, 67.17, 68.60, 69.45, 74.84, 81.49, 91.17, 101.04, 138.28, 150.36, 162.30; MS (CI-NH_3) m/e 634 ([M + NH_4^+], 2.3 %), 617 ([MH^+], 8.6), 375 ([MH^+ - $Me_3SiCH_2CH_3$], 55.2).

3-*N*-(Trimethylsilyl)ethoxymethyl-3',5'-*O*-(tetraisopropyldisilox ane-1,3-diyl)-2'-*O*-methyluridine 31

Alcohol **30** (27 mg, 0.044 mmol) was heated in methyl iodide (0.4 mL, 6.424 mmol) containing silver oxide (55 mg, 0.238 mmol) under reflux for 7 h. The reagent was removed *in vacuo* and the residue was chromatographed over silica gel (3:1 hexanes/ethyl acetate, v/v) giving **31** as an oil in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H, Si**Me**$_3$), 0.93 (t, 2H, Si**CH**$_2$), 1.02-1.07 (m, 28H, Si**CH**(**CH**$_3$)$_2$), 3.61 (s, 3H, 2'-O*Me*), 3.67 (t, 2H, Me_3SiCH_2**CH**$_2$O), 3.84 (m, 1H, H5'), 3.88 (m, 1H, H2'), 3.98 (m, 1H, H4'), 4.02 (m, 1H, H5"), 4.28 (dd, 1H, H3'), 5.34 (s, 2H, O**CH**$_2$N), 5.73 (d, 1H, H5), 5.82 (fine d, 1H, H1), 7.90 (d, 1H, H6), $J_{H1'-H2'}$ = 2.4 Hz, $J_{H2'-H3'}$ = 6.9, $J_{H3'-H4'}$ = 12.9, J_{H5-H6} = 8.2; ^{13}C NMR ($CDCl_3$) δ 12.30-13.44 (Me_2**C**HSi), 16.76-17.46 (**Me**$_2$CHSi and **Me**$_3$Si), 18.08 (Si**C**H$_2$), 59.08 (2'-O**Me**), 59.37 (C5'), 67.54 (SiCH$_2$**C**H$_2$), 68.17 (C3'), 69.74 (O**C**H$_2$N), 81.56 (C4'), 83.85 (C2'), 88.79 (C1'), 101.20 (C5), 138.02 (C6), 150.67 (C2), 162.68 (C4); MS (FAB-nitrobenzyl alcohol) m/e 653 ([M + Na^+], 3.0 %), 631 ([MH^+], 12.4), 573 (100), 529 ([MH^+ - $Me_3SiCH_2CH_3$], 74.7), 389 ([MH^+ - UracilH-3-N-$CH_2OCH_2CH_2SiMe_3$], 39.6), 357 (68.2); HRMS (FAB-glycerol), m/e calcd. for $C_{28}H_{55}N_2O_8Si_3$: 631.32692; found: 631.32663.

3-*N*-(Trimethylsilyl)ethoxymethyl-2'-*O*-methyluridine 32

Silyl ether **31** (305 mg, 0.484 mmol) was dissolved in THF (14.5 mL) and tetrabutylammonium fluoride (1.452 mL, 1.0 M solution in THF) was added at 0°C with stirring. After 15 min., the solvent was evaporated *in vacuo* and the residue chromatographed over silica gel (CH_2Cl_2 / MeOH 10:1) to afford **32** as a clear oil in quantitative yield: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.93 (t, 2H), 3.61 (s, 3H), 3.67 (t, 2H), 3.87 (q, 2H), 3.97-3.99 (m, 2H), 4.27 (q, 1H), 5.34 (s, 2H), 5.73 (d, 1H), 5.82 (fine d, 1H), 7.90 (d, 1H), $J_{H1'-H2'}$ = 2.4 Hz, J_{H5-H6} = 8.2; ^{13}C NMR ($CDCl_3$) δ 17.00, 18.07, 58.63, 60.65, 67.64, 68.12, 69.83, 83.18, 84.46, 89.18, 101.75, 139.52, 150.88, 162.75; MS (CI-NH_3) m/e 406 ([M + NH_4^+], 4.0 %), 389 ([MH^+], 5.5), 147 ([MH^+ - $Me_3SiCH_2CH_3$], 16.5).

3-*N*-(Trimethylsilyl)ethoxymethyl-2'-*O*-methyl-5'-*S*-acetyl-5'-deoxy-5'-thiouridine 33

Diisopropyl azodicarboxylate (0.194 mL, 0.969 mmol) was added dropwise to a stirred solution of triphenylphosphine (0.255 g, 0.969 mmol) in dry THF (3.7 mL) cooled to 0°C under nitrogen. After 0.5 h a solution of nucleoside **32** (0.188 g, 0.484 mmol) and thiolacetic acid (0.07 mL, 0.969 mmol) in dry THF (3.7 mL) was slowly added and the stirring at 0°C continued for 0.5 h after which time the reaction was allowed to warm to room temperature. After 30 min the solvent was removed *in vacuo* and the resulting yellow syrup was chromatographed over silica (3:2 hexanes / EtOAc) affording thiolester **33** as a white solid (0.134 g, 62 %). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H), 0.95 (t, 2H), 2.39 (s, 3H), 3.27 (A of ABX, 1H), 3.39 (B of ABX, 1H), 3.21-3.44 (m, 2H), 3.61 (s, 3H), 3.67 (t, 2H), 3.80 (m, 1H), 3.87 (m, 1H), 3.97-4.07 (m, 1H), 5.37 (fine d, 2H, J = 2.08 Hz), 5.82 (d, 1H), 5.80 (s, 1H), 7.48 (d, 1H), $^2J_{H5'A-H5'B}$ = -14.4, $J_{H4';-H5'B}$ = 4.2, $J_{H4'-H5'A}$ = 6.6, J_{H5-H6} = 8.2; ^{13}C NMR ($CDCl_3$) δ 18.14, 30.54, 37.16, 58.70, 67.66, 69.91, 71.98, 81.78, 83.25, 89.02, 102.18, 137.97, 150.75, 162.35, 194.70; MS (FAB-nitrobenzyl alcohol) m/e 469 ([M + Na^+], 7.3 %), 447 ([MH^+], 7.3), 419 (100), 345 (3.3), 279 (100), 205 ([MH^+ - UracilH-3-N-$CH_2OCH_2CH_2SiMe_3$], 38.6); HRMS (FAB-glycerol), m/e calcd. for $C_{18}H_{31}N_2O_7SSi$: 447.16207; found: 447.16213.

3-*N*-(Trimethylsilyl)ethoxymethyl-2'-*O*-methyl-5'-deoxy-5'-thiouridine 34

A solution of thiolester **33** (455 mg, 1.05 mmol) in dry methanol (5 mL) previously saturated with nitrogen was cooled to 0°C and a stream of ammonia gas passed through for 15 min. The ice bath was removed and the reaction stirred for 20 h. The solvent was then evaporated *in vacuo* yielding a white solid which was chromatographed over silica (4:1 EtOAc / hexanes, v/v) to afford the thiol **34** as a white solid (410 mg, 98 % yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.00 (s, 9H, Si**Me**$_3$), 0.95 (t, 2H, SiC**H**$_2$), 1.57 (t, 1H, S**H**), 2.88 (A of ABXY, 1H, H5'$_A$), 3.05 (B of ABXY, 1H, H5'$_B$), 3.62 (s, 3H, 2'-**OMe**), 3.67 (t, 2H, Me_3SiCH_2C**H**$_2$O), 3.79 (q, 1H, H4'), 3.95-4.10 (m, 2H, H2' and H3'), 5.38 (fine d, 2H, OC**H**$_2$N, J = 1.6 Hz), 5.80 (d, 1H, H5), 5.89 (fine d, 1H, H1'), 7.63 (d, 1H, H6), $J_{H1'-H2'}$ = 1.6 Hz, J_{H5-H6} = 8.2, $^2J_{H5'A-H5'B}$ = -14.6, $J_{H4';-H5'B}$ = 3.5, $J_{H4'-H5'A}$ = 4.6, $J_{SH-H5'A}$ = 8.5, $J_{SH-H5'B}$ = 8.9; ^{13}C NMR ($CDCl_3$) δ 18.14 (Si**C**H$_2$), 25.87 (C5'), 58.88 (2'-O**Me**), 67.64 (SiCH$_2$**C**H$_2$), 69.93 (O**C**H$_2$N), 70.60 (C3'), 82.69 (C4'), 83.33 (C2'), 88.66 (C1'), 102.25 (C5), 138.20 (C6), 150.68 (C2), 162.32 (C4),; MS (FAB-nitrobenzyl alcohol) m/e 427 ([M + Na^+], 9.6 %), 405 ([MH^+], 66.6), 347 (100), 303 ([MH^+ - $Me_3SiCH_2CH_3$], 29.3); HRMS (FAB-glycerol), m/e calcd. for $C_{16}H_{29}N_2O_6SSi$: 405.15146; found: 405.15156.

Disulfide of thiol **34**: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.00 (s, 18H), 0.93 (t, 4H), 2.99 (A of ABX, 2H), 3.29 (B of ABX, 2H), 3.59 (s, 6H), 3.65 (t, 4H), 3.83 (dd, 2H),

3.93-4.16 (m, 4H), 5.34 (fine d, 4H, J = 1.6 Hz), 5.77 (d, 2H), 5.80 (s, 2H), 7.38 (d, 2H), $J_{H5\text{-}H6}$ = 8.2, $^2J_{H5'A\text{-}H5'B}$ = -14.3, $J_{H4'\text{-}H5'B}$ = 3.2, $J_{H4'\text{-}H5'A}$ = 6.7; MS (FAB - nitrobenzyl alcohol) m/e 829 ([M + Na^+}, 2.5 %), 807 ([MH^+], 3.1), 645, (39.6), 515 (31.9), 403 (21.4).

Reduction of disulfide to 34

A solution of the disulfide of **34** (57 mg, 0.071 mmol) and PPh_3 (18 mg, 0.075 mmol) in a mixture of dioxane (0.4 ml) and water (0.1 ml), containing one drop of conc. HCl, was stirred under nitrogen for 1 hour. The solvent was removed *in vacuo* and the residue taken up in ether and dried with magnesium sulfate. The residue was chromatographed over silica gel (10:1 CH_2Cl_2 / MeOH) to give **34** as a colorless glass (50 mg, 87 % yield).

Sulfide linked thymidine / 2'-*O*-methyluridine dimer 39

Cesium carbonate (61 mg, 0.19 mmol), previously flame dried *in vacuo*, was suspended in dry DMF (0.8 mL) and a solution of mesylate **38** (29) (57 mg, 0.12 mmol) and thiol **34** (55 mg, 0.14 mmol) in dry DMF (1.3 mL) was then added resulting in a yellow solution. After 3 h of stirring at ambient temperature under a nitrogen atmosphere, the solvent was removed *in vacuo* and the product was extracted with dichloromethane (50 + 30 mL) and washed with aqueous sodium bicarbonate (5 % w/v, 20 mL) and water (20 mL). The combined organic phases were dried (Na_2SO_4), filtered and evaporated *in vacuo* yielding a yellow foam. Chromatography over silica gel (4:1 EtOAc / hexanes, v/v) afforded the sulfide **39** as a colorless solid (81 mg, 85 % yield): ^1H NMR (200 MHz, $CDCl_3$) δ 0.02 (s, 9H), 0.10 (s,6H), 0.88-0.99 (m, 11H), 1.45-1.85 (m, 2H), 1.90 (s, 3H), 2.05-2.52 (m, 3H), 2.56-2,66 (m, 2H), 2.75-2.86 (dd, 2H), 3.57 (s, 3H), 3.70(t 2H), 3.69-3.76 (m, 2H), 3.94-4.03 (m, 2H), 4.27 (t, 1H), 5.35 (s, 2H), 5.79 (d, 1H), 5.88 (fine d, 1H), 6.08 (dd, 1H), 7.39 (d, 1H), 7.56 (q, 1H), 8.57 (s, 1H); ^{13}C-NMR (75.4 MHz, $CDCl_3$) δ 13.24, 18.07-18.38, 25.73, 32.27, 32.90, 34.52, 38.85, 41.65, 58.87, 62.86, 67.60, 69.88, 71.66, 81.66, 83.00, 85.93, 88.88, 89.66, 102.25, 110.02, 138.34, 150.59, 150.72, 162.25, 163.48; MS (FAB - nitrobenzyl alcohol) m/e 793 ([M + Na^+}, 1.4 %), 771 ([MH^+], 3.7), 645 ([MH^+ - ThyH], 45.9), 403 ([MH+ - ThyH - UraH-3-N-SEM], 19.6).

Sulfide linked thymidine / 2'-*O*-methyluridine dimer 40

A solution of tetra-*n*-butylammonium fluoride in THF (1 M, 0.8 mL, 0.80 mmol) was added to a stirred solution of sulfide **39** (47 mg, 0.06 mmol) in dry THF (0.6 mL). After 10 min the solution was evaporated *in vacuo* and the resulting glass was

chromatographed over silica gel (10:1 CH_2Cl_2 / MeOH) to give **40** as a colorless glass in quantitative yield: ^{1}H NMR (200 MHz, CD_3OD) δ 0.02 (s, 9H, ^{3}Si**Me**$_3$), 0.87-0.95 (t, 2H, ^{3}SiC**H**$_2$, *J* = 8.1 Hz), 1.24-1.65 (m, 2H, ^{5}H1"$_{AB}$), 1.86 (s, 3H, ^{5}5-**Me**), 2.05-2.44 (m, 3H, ^{5}H2' and ^{5}H3'), 2,66 (t, 2H, ^{5}H2"), 2.89-2.96 (dd, 2H, ^{3}H5'), 3.52 (s, 3H, ^{3}2'-O**Me**), 3.67(t 2H, ^{3}SiCH$_2$C**H**$_2$O), 3.72 (m, 2H, ^{3}H4' and ^{5}H5'$_A$), 3.87 (m, 2H, ^{5}H4' and ^{5}H5'$_B$), 4.09 (m, 2H, ^{3}H2' and ^{3}H3'), 5.34 (s, 2H, ^{3}NC**H**$_2$O), 5.81 (d, 1H, ^{3}H6), 5.88 (fine d, 1H, ^{3}H1', *J* = 2.8), 6.04 (m, 1H, ^{5}H1'), 7.80 (d, 1H, ^{3}H5), 7.97 (s, 1H, ^{5}H6); ^{13}C-NMR (75.4 MHz, CD_3OD) δ 12.53 (^{5}5-**Me**), 18.89 (^{3}Si**Me**$_3$, ^{3}Si**C**H$_2$CH$_2$O), 32.28 (^{3}C5'), 33.07 (^{5}C2"), 34.58 (^{5}C1"), 37.50 (^{5}C3'), 39.86 (^{5}C2'), 59.01 (^{3}2'-O**C**H$_3$), 62.13 (^{5}C5'), 68.51 (^{3}SiCH$_2$**C**H$_2$O), 71.09 (^{3}O**C**H$_2$N), 72.69 (^{3}C3'), 84.21 (^{3}C4'), 84.60 (^{3}C2'), 86.38 (^{5}C1'), 87.89 (^{3}C1'), 90.47 (^{5}C4'), 102.46 (^{3}C5), 110.70 (^{5}C5), 138.41 and 141.29 (^{5}C6, ^{3}C6), 1152.27 (^{3}C2 and ^{5}C2), 157.69 (^{3}C4), 164.64 (^{5}C4); MS (FAB - nitrobenzyl alcohol) m/e 679 ([M + Na$^+$}, 11.1 %), 657 ([MH$^+$], 37.1), 531 ([MH$^+$ - ThyH], 100), 289 ([MH$^+$ - ThyH - UraH-3-N-SEM], 83.3), HRMS (FAB-glycerol), m/e calcd. for $C_{28}H_{45}N_4O_{10}SSi$: 657.26251; found: 657.26257.

Sulfide linked thymidine / 2'-*O*-methyluridine dimer 41

4,4'-Dimethoxytrityl chloride (32 mg, 0.09 mmol) and dimer **40** (49 mg, 0.075 mmol) in dry pyridine (0.8 mL) containing dimethylaminopyridine (2 mg, 0.01 mmol) and triethylamine (0.04 mL) were stirred for 8 h, the reaction mixture was poured into water (25 mL) and extracted with dichloromethane (3 x 15 mL). The organic phases were dried over Na_2SO_4 and evaporated *in vacuo* to a syrup which was then chromatographed over silica gel (100:5:10 CH_2Cl_2 / MeOH / Et^{3}N, v/v) giving **41** as a white foam (58 mg, 82 % yield): $_1$H NMR (300 MHz, acetone-d6) δ 0.02 (s, 9H), 0.86-0.91 (t, 2H, *J* = 8.2 Hz), 1.58-1.79 (m, 2H), 1.49 (s, 3H), 2.13-2.40 (m, 3H), 2,55-2.67 (m, 2H), 2.83-2.89 (dd, 1H), 2.95-3.01 (dd, 1H), 3.10-3.53 (m, 3H), 3.49 (s, 3H), 3.66(t 2H), 3.78 (s, 6H), 3.93 (q, 1H), 4.06 (dd, 1H), 4.15 (dd, 1H), 5.29 (s, 2H), 5.67 (d, 1H), 5.92 (fine d, 1H), 6.08 (dd, 1H), 6.88-7.52 (m, 13H), 7.70 (s, 1H), 7.72 (d, 1H), $J_{5H1'\text{-}5H2'A}$ = 7.1, $J_{5H1'\text{-}5H2'B}$ = 3.2, $J_{3H1'\text{-}3H2'}$ = 3.4, $J_{3H2'\text{-}3H3'}$ = 5.4, $J_{3H3'\text{-}3H4'}$ = 6.3, $J_{3H4'\text{-}3H5'B}$ = 4.5, $J_{3H4'\text{-}3H5'A}$ = 6.2, $^2J_{3H5'A\text{-}3H5'B}$ = -14.2, $J_{3H5\text{-}3H6}$ = 8.2; ^{13}C-NMR (75.4 MHz, Acetone-d$_6$) ar and Ph in the parentheses indicate the C_6H_5- and $MeOC_4H_4$- in DMTr, δ 12.36 (^{5}5-**Me**), 18.57 (^{3}Si**Me**$_3$, ^{3}Si**C**H$_2$CH$_2$O), 31.81 (^{3}C5'), 32.84 (^{5}C2"), 34.52 (^{5}C1"), 38.12 (^{5}C3'), 39.27 (^{5}C2'), 55.54 (O**Me**$_3$ in ar), 58.75 (^{3}2'-O**Me**$_3$), 64.16 (^{5}C5'), 67.84 (^{3}SiCH$_2$**C**H$_2$O), 70.48 (^{3}O**C**H$_2$N), 72.47 (^{3}C3'), 83.72 (^{3}C4'), 84.32 (^{3}C2'), 85.49 (^{5}C1'), 85.59 (^{3}C1'), 87.17 (Ph(MeOPh)$_2$**C** in ar), 89.58 (^{5}C4'), 102.31 (^{3}C5), 110.27 (^{5}C5), 113.97 (**C**H-C-OMe in ar), 127.67 (**C**HCHCH-C- in Ph), 128.66 (**C**H-C- of Ph), 129.04 (**C**HCH-C- in Ph), 131.00 (**C**HCH-C-OMe in ar), 136.51 (^{3}C6), 136.63 (-**C**-

CHCHCOMe in ar), 140.10 (^{5}C6), 145.92 (-**C**-CHCHCH in Ph), 151.20 and 151.81 (^{3}C2 and ^{5}C2), 159.68 (^{3}C4), 162.78 (CH-**C**-OMe in ar), 164.32 (^{5}C4); MS (FAB - nitrobenzyl alcohol) m/e 981 ([M + Na$^+$}, 16.1 %), 959 ([MH$^+$], 13.7), 833 ([MH$^+$ - ThyH], 14.3), 655 ([MH$^+$ - DMTrH], 14.3).

Sulfide linked thymidine / 2'-*O*-methyluridine dimer 42

Dimer **41** (32mg, 0.033 mmol) was dissolved in THF (0.5 ml) containing triethylamine (0.1 ml) tetrabutylammonium fluoride. After refluxing for 20 hrs, the solvent was removed *in vacuo* and the residue was then chromatographed over silica gel (100:3:10 CH_2Cl_2 / MeOH / Et_3N, v/v) giving **42** as a white glass (27 mg, 98 % yield): ^{1}H NMR (300 MHz, CD_3OD) δ 1.51-1.64 (m, 2H), 1.84 (s, 3H), 1.99-2.31 (m, 3H), 2,47-2.66 (m, 2H), 2.70-2.77 (dd, 1H), 2.80-2.86 (dd, 1H), 3.10-3.47 (m, 3H), 3.42 (s, 3H), 3.70 (s, 6H), 3.77 (dd, 1H), 3.94 (dt, 1H), 4.02 (dd, 1H), 5.51 (d, 1H), 5.77 (fine d, 1H), 5.97 (dd, 1H), 6.76-7.40 (m, 13H), 7.56 (d, 1H), 7.76 (s, 1H), $J_{5H1'\text{-}5H2'A}$ = 7.3, $J_{5H1'\text{-}5H2'B}$ = 2.9, $J_{3H1'\text{-}3H2'}$ = 3.4, $J_{3H2'\text{-}3H3'}$ = 5.4, $J_{3H3'\text{-}3H4'}$ = 6.8, $J_{3H4'\text{-}3H5'B}$ = 4.4, $J_{3H4'\text{-}3H5'A}$ = 5.9, $^2J_{3H5'A\text{-}3H5'B}$ = -14.4, $J_{3H5\text{-}3H6}$ = 8.1; ^{13}C-NMR (75.4 MHz, CD_3OD) δ 32.24, 34.70, 37.63, 37.66, 39.96, 55.79, 58.97, 63.76, 72.67, 84.06, 84.53, 86.64, 86.84, 87.80, 89.69, 103.02, 110.97, 114.16, 128.10, 128.95, 129.36, 131.44, 136.80, 137.86, 142.25, 146.03, 151.88, 152.26, 160.29, 164.79, 165.86; MS (FAB - nitrobenzyl alcohol) m/e 829 ([MH$^+$], 6.3 %), 703 ([MH$^+$ - ThyH], 5.0), 655 ([MH$^+$ - ThyH - UraH], 5.9); HRMS (FAB - glycerol) m/e calcd. for $C_{43}H_{49}N_4O_{11}S$ 829.31184, found: 829.31186.

Sulfide linked thymidine / 2'-*O*-methyluridine dimer 43

2-Cyanoethyl *N,N*-diisopropylchlorophophoramidite (33 μL, 0.142 mmol) was slowly added to a stirred solution of tritylated dimer **42** (61 mg, 0.071 mmol) in dry CH_2Cl_2 (0.5 mL) containing NEt_3 (49 mL, 0.284 mmol). After 20 h of stirring under nitrogen, the solution was quenched with methanol (0.1 mL) and the solvent was removed *in vacuo*. The residue was chromatographed over silica gel (100:5:1 CH_2Cl_2 / MeOH / Et_3N, v/v) giving the phosphoramidite **43** as a colorless foam in quantitative yield (73 mg) which was used as such in the subsequent solid-phase syntheses: ^{1}H NMR (200 MHz, CD_3OD) δ 1.26 and 1.29 (d, NCH(C**H**$_3$)$_2$), 1.45-1.62 (m, 2H, ^{5}H1"$_{AB}$), 1.82 (s, 3H, ^{5}5-**Me**), 1.95-2.32 (m, 3H, ^{5}H2'$_{AB}$ and ^{5}H3'), 2,46-2.69 (m, 2H, ^{5}H2"), 2.51 (t, 2H, C**H**$_2$CN), 2.70-2.88 (m, 2H, ^{3}H5'), 2.92-3.47 (m, 3H, ^{5}H5' and ^{5}H4'), 3.42 (s, 3H, ^{3}2'-**OMe**), 3.67 (t, 2H, POC**H**$_2$), 3.68 (s, 6H, 2 X OMe), 3.87-4.12 (m, 3H, ^{3}H2', ^{3}H3', ^{3}H4'), 5.48 (d, 1H, ^{3}H6), 5.80 (fine d, 1H, ^{3}H1'), 5.95 (dd, 1H, ^{5}H1'), 6.75-7.39 (m, 13H, DMT), 7.53 (d, 1H, ^{3}H5), 7.74 (s, 1H, ^{5}H6), $J_{5H1'\text{-}5H2'A}$ = 6.9, $J_{5H1'\text{-}5H2'B}$ = 2.5,

$J_{3H1'-3H2'}$ = 4.8, $J_{3H5-3H6}$ = 8.1; ^{13}C-NMR (75.4 MHz, CD_3OD) δ 19.32 and 19.36 (NCH**Me**$_2$) 32.48 (^{3}C5'), 34.55 (^{5}C1"), 37.58 (^{5}C3'), 39.99 (^{5}C2'), 46.70 (N**C**HMe$_2$) 55.73 (O**Me**$_3$ in ar), 58.96 (^{3}2'-O**Me**$_3$), 63.45 and 63.74 (^{5}C5' and ^{5}C2"), 72.75 (^{3}C3'), 84.06 and 84.09 (^{3}C4' and ^{3}C2'), 86.53 (^{5}C1' and ^{3}C1'), 87.82 (Ph(MeOPh)$_2$**C** in ar), 89.65 (^{5}C4'), 103.00 (^{3}C5), 110.97 (^{5}C5), 114.32 (**C**H-C-OMe in ar), 128.75 (**C**HCHCH-C- in Ph), 128.95 (**C**H-C- of Ph), 129.57 (**C**HCH-C- in Ph), 131.37 (**C**HCH-C-OMe in ar), 136.78 (^{3}C6), 137.82 (-**C**-CHCHCOMe in ar), 142.33 (^{5}C6), 146.02 (-**C**-CHCHCH in Ph), 152.26 (^{3}C2 and ^{5}C2), 160.30 (^{3}C4), 163.26 (CH-**C**-OMe in ar), 165.04 (^{5}C4); ^{31}P-NMR (acetone) δ 150.59 and 150.95 ppm.

2'-*O*-Methyl-5'-*S*-acetyl-5'-deoxy-5'-thiouridine 36

Diisopropyl azodicarboxylate (1.53 ml, 7.80 mmole) was added dropwise to a cooled (0°C) solution of triphenylphosphine (2.05 g, 7.84 mmole) in dry THF (29 ml) resulting in a milky suspension. After 0.5 h, a solution of diol **35** (1.00 g, 3.88 mmole) was slowly added to the reaction mixture. It was stirred at 0°C for 3 h and the solvent removed in *vacuo*. Chromatography over silica gel (10 : 1 CH_2Cl_2 / MeOH, v/v) afforded thiolester **36** (832 mg, 82%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 2.39 (s, 3H), 3.25 (A of ABX, 1H), 3.32 (B of ABX, 1H), 3.55 (s, 3H), 3.76-4.06 (m, 3H), 5.80 (overlapping s and d, 2H), 7.45 (d, 1H), 10.05 (broad s, 1H), $J_{H4'-H5'A}$ = 6.6, $J_{H4'-H5'B}$ = 4.2, $^2J_{H5'A-H5'B}$ = -12, J_{H5-H6} = 8.2; ^{13}C-NMR ($CDCl_3$) δ 30.59, 58.6, 71.78, 81.83, 83.08, 88.39, 102.65, 138.79, 150.11, 183.63, 195.84; MS (CI-NH_3) m/e 317 ([MH^+], 45.7 %), 247 (22.1), 241 ([MH^+ - CH_3COSH], 19.9), 113 ([UraH + H^+], 100).

2'-*O*-Methyl-5'-deoxy-5'-thiouridine 37

Thiolacetate **36** (518 mg, 1.64 mmole) was dissolved in MeOH (15 ml) cooled in an ice-bath and NH_3 gas was bubbled into the solution under iced-bath for 10 min. The solution was stirred for 3 h at room temperature and the solvent was removed. The residue was purified on a silica gel column (20:1 CH_2Cl_2/MeOH, v/v) and yielded thiol **37** (391 mg, 87%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.80 (t, 1H, SH), 2.86 (A of ABXY, 1H, $H5'_A$), 3.00 (B of ABXY, 1H, $H5'_B$), 3.58 (s, 3H, 2'-OMe), 3.81 (dd, 1H, H2'), 4.01 (dt, 1H, H4'), 4.10 (m, 1H, H3'), 5.77 (d, 1H, H5), 5.88 (d, 1H, H1'), 7.63 (d, 1H, H6'), 10.20 (broad s, 1H, NH), $J_{H1'-H2'}$ = 2.1, $J_{H2'-H3'}$ = 5.6, $J_{H3'-H4'}$ = 7.4, $J_{H4'-H5'A}$ = 4.5, $J_{H4'-H5'B}$ = 3.9, $^2J_{H5'A-H5'B}$ = -13.2, $J_{SH-H5'A}$ = 8.5, $J_{SH-H5'B}$ = 8.8, J_{H5-H6} = 8.1; ^{13}C-NMR ($CDCl_3$) δ 25.88 (C5'), 58.73 (2'-OMe), 70.38 (C3'), 82.37 (C2'), 83.07 (C4'), 87.87 (C1'), 102.52 (C5), 139.99 (C6), 150.12 (C2), 163.68 (C4); MS (CI-NH_3) m/e 275 ([MH^+], 60.3%), 113 ([UraH = H^+], 100), HRMS (CI-NH_3) m/e calcd. for $C_{10}H_{15}N_2O_5S$: 275.07018, found: 275.07005.

Sulfide linked 2'-*O*-methylthymidine/2'-*O*-methyluridine dimer 44

Cesium carbonate (70 mg, 0.42mmol), previously flame dried in vacuo, was suspended in dry DMF (1.3 ml) and a deoxygenated solution (saturated with N_2 over 20 min.) of mesylate **16** (89 mg, 0.15 mmol) and thiol **37** (60 mg, 0.22 mmol) in dry DMF (2 ml) was then added, resulting in a yellow solution. After 1 h of stirring at room temperature under nitrogen, the solvent was removed and the product was dissolved in CH_2Cl_2 (2 x 15 ml) and washed with 5% aq. $NaHCO_3$ (5 ml) and water (5 ml). The combined organic layers were dried (Na_2SO_4) and evaporated, yielding a yellow foam. Chromatography over silica gel (1 : 2.5 EtOAc / hexanes, v/v) afforded the dimer **44** (94 mg, 80%), m.p. 97-98^{0}C (EtOH / H_2O): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.07 (s, 9H), 1.44 (s, 3H), 1.70-1.91 (m, 2H), 2.31 (m, 1H), 2.47-2.63 (m, 2H), 2.75-3.08 (m, 2H), 3.53, 3.56 (two s, 6H), 3.71-3.81 (m, 3H), 3.91-4.22 (m, 4H), 5.71 (d, 1H, ^{3}H5), 5.86 (s, 2H), 7.72-7.66 (m, 11H), 9.91, 10.09 (two br s, 2H), $J_{5H1'-5H2'}$ = 0, $J_{3H1'-3H2'}$ = 0, $J_{3H5-3H6}$ = 8.0; ^{13}C-NMR ($CDCl_3$) δ 11.93, 19.46, 24.08, 27.00, 31.48, 33.28, 39.82, 58.07, 58.78, 62.49, 70.99, 82.33, 83.21, 84.87, 85.15, 88.13, 88.67, 102.63, 110.39, 127.87-139.78 (Ph and 2 x C6), 150.05, 150.41, 164.01, 164.34; MS (FAB-NBA) m/e 795 ([MH$^+$], 13.9%), 669 ([MH$^+$ - ThyH], 100), 521 (16.9), 369 (23.1), 315 (54.3); HRMS (FAB-Glycerol) m/e calcd. for $C_{39}H_{51}N_4O_{10}SSi$: 795.30952, found: 795.30957.

Sulfide linked 2'-*O*-methylthymidine/2'-*O*-methyluridine dimer 45

A solution of tetra-butylammonium fluoride in THF (1M, 0.22 ml, 0.22 mmole) was added to a solution of dimer **44** (90 mg, 0.11 mmole) in dry THF (0.75 ml). After 3 h the solution was evaporated and the resulting syrup was chromatographed over silica gel (20:1 CH_2Cl_2/MeOH, v/v) giving diol **45** (51 mg, 81%), m.p. 148-151^{0}C (CH_2Cl_2 / hex): ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.43-1.68 (m, 2H), 1.74 (s, 3H), 2.18 (H^7, 1H), 2.40-2.58 (m, 2H), 2.72-2.78 (m, 2H), 3.41 (s, 6H), 3.59-3.70 (m, 3H), 3.82-3.96 (m, 4H), 5.61 (d, 1H), 5.72 (d, 1H) 5.74 (s, 1H), 7.53 (d, 1H), 7.99 (s, 1H). $J_{3H5-3H6}$ = 8.0; ^{13}C-NMR ($CDCl_3$) δ 12.49, 24.32, 31.66, 33.66, 39.19, 58.22, 58.95, 59.99, 71.34, 82.75, 83.52, 85.71, 85.90, 88.70, 88.82, 102.70, 110.01, 137.07, 140.80, 150.62, 150.91, 164.60, 165.35; MS (FAB-NBA) m/e 557 ([MH$^+$], 26.9%), 431 ([MH$^+$ - ThyH], 44.9), 307 (31.2), 195 (40.4), 186 (100), 128 ([ThyH + 2H$^+$], 100), 113 ([UraH + H$^+$], 30.4); HRMS (FAB-Glycerol) calcd. for $C_{23}H_{33}N_4O_{10}S$: 557.19174, found, 557.19149.

Sulfide linked 2'-*O*-methylthymidine/2'-*O*-methyluridine dimer 46

4,4-Dimethoxytrityl chloride (80 mg, 0.24 mmole) was added to a solution of diol **45** (44 mg, 0.08 mmole) in dry pyridine (1.10 ml) and triethylamine (0.06 ml, 0.39 mmole) at room temperature. After 10 h the solvent was removed and the resulting syrup

was chromatographed over silica gel (100 : 5 : 1 CH_2Cl_2 / MeOH / Et_3N, v/v) giving **46** (56 mg, 83%), m.p. 84-86^{0}C (EtOH /H_2O): ^{1}H-NMR (200 MHz, acetone) δ 1.40 (m, 2H), 2.02 (s, 3H), 2.58-2.94 (m, 5H), 3.24 (m, 1H), 3.48 and 3.61 (two s, 6H), 3.65 (m, 1H), 3.79 (s, 6H), 3.92-4.31 (m, 4H), 5.56 (d, 1H), 5.86 (s, 1H), 5.89 (d, 1H), 6.85-7.51 (m, 9H), 7.68 (d, 1H), 7.81 (s, 1H), 10.17 (br s, 2H), $J_{5H1'-5H2'}$ = 0, $J_{3H1'-3H2'}$= 5.1, $J_{3H5-3H6}$ = 8.2; ^{13}C-NMR (acetone) δ 11.92 ppm , 26.18, 30.69, 34.84, 39.81, 40.97, 46.34, 55.49, 58.45, 62.60, 73.91, 84.65, 85.15, 86.31, 87.15, 109.99, 110.90, 127.20-145.56 (Ph and 2 x C6), 151.05, 151.21, 159.67, 164.36; MS (FAB-NBA) m/e 944 ([M + Et_3NH^+], 4.7 %), 460 (10.1), 391 (16.5), 303 ([DMTrH], 100); HRMS (FAB-Glycerol) m/e calcd. for $C_{44}H_{51}N_4O_{12}S_1$ (MH^+): 859.32242, found: 859.32257.

Sulfide linked 2'-*O*-methylthymidine/2'-*O*-methyluridine dimer 47

2-Cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.100 mL, 0.45 mmol) was slowly added to a stirred solution of tritylated alcohol **46** (195 mg, 0.22 mmol) in dry CH_2Cl_2 (1 mL) containing NEt_3 (0.077 mL, 0.52 mmol). After 14 h of stirring at room temperature under nitrogen, the solvent was evaporated yielding a pale yellow foam. The crude product was chromatographed over silica gel (100 : 5 : 1 CH_2Cl_2 / MeOH / Et_3N, v/v) affording the phosphoramidite **47** as a colorless foam (205 mg, 86%), which was used as such in the subsequent solid-phase syntheses: ^{1}H-NMR (200 MHz, acetone) δ 1.08-1.25 (m, 12H, -N(CH**Me**$_2$)$_2$) 1.42 (m, 1H, ^{5}H1$_A$"), 1.83 (m, 1H, ^{5}H1$_B$"), 2.07 (s, 3H, 5-Me), 2.58-2.95 (m, 9H, ^{5}H3', ^{3}H2', ^{5}H2", ^{3}H5' and -CH_2CN), 3.26 (m, 1H, ^{5}H2'), 3.52 and 3.55 (2s, 6H, 2 x 2-OMe), 3.82 (s, 6h, 2 x PhOMe), 3.60-4.43 (m, 9H, ^{5}H5',^{3}H4', ^{5}H4', ^{3}H3', -N(**C**H_2Me$_2$)$_2$ and -$POCH_2$), 5.80 (m, 1H, ^{5}H1'), 6.97 (m, 1H, ^{3}H1'), 6.90-7.81 (m, 15H, DMTr and 2 x H6), 10.08 (s, 2H, 2 x NH); ^{31}P-NMR (81.0 MHz, acetone) δ 152.61 and 152.27 ppm; MS (FAB - Glycerol / NBA) m/e 1059 ([MH^+], 12.8%), 988 (19.0), 933 ([MH^+ - ThyH], 7.2), 841 ([MH+ - *i*Pr^{2}NH$^+$P(OH)OCH$_2$CH$_2$CN], 5.5), 689 (18.8), 474 (100).

Sulfone Diol 48

Diol **19a** (92 mg, 0.180 mmol) was dissolved in MeOH (5.0 ml) and cooled to 0^{0}C. To this was added a water solution of oxone (0.06 mmol/ml, pH=6.4 phosphate buffer, 9.0 ml). The resulting slurry was stirred for 2 h at room temperature. Evaporation yielded a colorless residue, which was chromatographed over silica gel (5:1 CH_2Cl_2 / MeOH) to give **30** as a white powder (66 mg. 68% yield): ^{1}H NMR (200 MHz, CD_3OD) δ 1.34-1.46 (m, 1H, ^{5}H1"$_A$), 1.58-1.74 (m, 1H, ^{5}H1"$_B$), 1.85 and 1.87 (two s, 6H, 5-**Me**), 1.94-2.38 (m, 4H, ^{5}H2'$_{AB}$ and ^{3}H2'$_{AB}$), 2,37-2.56 (m, 1H, ^{5}H3'), 3.05-3.27 (m, 4H, ^{5}H2"$_{AB}$ and ^{3}H5'), 3.58-3.73 (m, 2H, ^{5}H4' and ^{5}H5'$_A$), 3.78-3.87 (m, 1H,

^{5}H5'$_B$), 4.17-4.26 (m, 1H, ^{3}H4'), 4.35-4.43 (dt, 1H, ^{3}H3'), 6.01 (dd, 1H, ^{5}H1'), 6.14 (t, 1H, ^{3}H1'), 7.48 and 7.89 (two s, 2H, H6), $J_{5H1'\text{-}5H2'A}$ = 6.7, $J_{5H1'\text{-}5H2'B}$ = 3.7, $J_{3H1'\text{-}3H2'}$ = 6.7, $J_{3H2'A\text{-}3H3'}$ = 6.8, $J_{3H2'B\text{-}3H3'}$ = 4.6; MS (FAB - nitrobenzyl alcohol) m/e 564 ([M + Na$^+$], 5.9%), 543 ([MH$^+$], 15.8), 417 ([MH$^+$ - ThyH], 17.7), 307 (100).

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