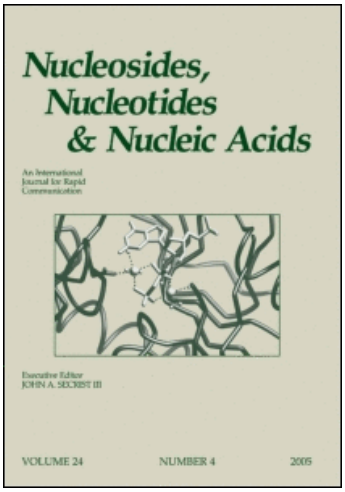



**Nucleosides, Nucleotides and Nucleic Acids**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

# Synthesis of 2'-Substituted MMI Linked Nucleosidic Dimers: An Optimization Study in Search of High Affinity Oligonucleotides for Use in Antisense Constructs

Didier Peoc'h[a]; Eric E. Swayze[a]; Balkrishen Bhat[a]; Yogesh S. Sanghvi[a]
[a] Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc., Carlsbad, California, USA

Online publication date: 02 October 2004

**To cite this Article** Peoc'h, Didier , Swayze, Eric E. , Bhat, Balkrishen and Sanghvi, Yogesh S.(2004) 'Synthesis of 2'-Substituted MMI Linked Nucleosidic Dimers: An Optimization Study in Search of High Affinity Oligonucleotides for Use in Antisense Constructs ', Nucleosides, Nucleotides and Nucleic Acids, 23: 1, 411 — 438
**To link to this Article: DOI:** 10.1081/NCN-120028337
**URL:** http://dx.doi.org/10.1081/NCN-120028337

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 23, Nos. 1 & 2, pp. 411–438, 2004

# Synthesis of 2′-Substituted MMI Linked Nucleosidic Dimers: An Optimization Study in Search of High Affinity Oligonucleotides for Use in Antisense Constructs[†]

**Didier Peoc'h, Eric E. Swayze,[*] Balkrishen Bhat, and Yogesh S. Sanghvi**

Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc.,
Carlsbad, California, USA



## ABSTRACT

The synthesis of a series of methylene(methylimino) (MMI) linked oligodeoxyribonucleotide dimers modified at the 2′-position with fluoro and/or methoxy groups and their incorporation into different sequences has been accomplished. From these dimers, bis 2′-OMe MMI dimer was selected for further studies based on its synthetic accessibility and the increased thermodynamic stability conferred upon oligonucleotides incorporating this modification.

*Key Words:* Antisense; Oligonucleotide; MMI dimer.

[†]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

[*]Correspondence: Eric E. Swayze, Department of Medicinal Chemistry, Isis Pharmaceuticals, Inc., 2292 Faraday Ave., Carlsbad, CA 92008, USA; Fax: (760) 603-4653; E-mail: eswayze@isisph.com.



DOI: 10.1081/NCN-120028337 1525-7770 (Print); 1532-2335 (Online)

## INTRODUCTION

Replacement of the natural phosphodiester linkage of nucleic acids with a nonionic dephosphono linkage has proven to be a useful strategy for the preparation of antisense oligomers exhibiting enhanced nucleolytic stability and higher binding affinity for the complement RNA.[1–4] The prospects of improved cellular uptake with oligomeric constructs that are more lipophilic and carry reduced net negative charge than natural DNA and RNA has provided further stimulation for the synthesis of such surrogates.[5] Our investigations in this area have resulted in the discovery of the *m*ethylene (*m*ethyl*i*mimo) (MMI) linkage, which we have found to be an excellent backbone modification, as it confers several desirable attributes on to the resulting antisense oligomer.[6]

In addition to the modification of 3′ → 5′ linkages, modifications at the 2′-position of nucleic acids with a variety of substituents have been successfully utilized in antisense strategies leading to oligomeric analogs with improved affinity.[7] In certain cases the improved affinity has translated well into increased biological activity in cell based experiments, as well as higher efficacy in animals.[8] Therefore, a logical choice was to combine one of our best backbone modifications (i.e. MMI) with an appropriate 2′-sugar substituent to create oligomers that may exhibit higher affinity towards their corresponding RNA target, as well as generally improved antisense properties.

Damha et al.,[9] Grayznov et al.,[10,11] Reynolds et al.[12] and DeMesmaeker et al.[13] have independently published on such additive and selective effects on the affinity for complement RNA over DNA, in which a 2′-substituent was added to a backbone modified oligomer. However we believe that a systematic optimization of the 2′-substituents has never been attempted with a series of suitable functionalities in combination with a single nonionic backbone modification. We elected the 2′-F, 2′-OMe, 2′-O$(CH_2)_2$OMe as the suitable 2′-functionalities for our studies based on their high affinity profile.[14] Herein, we describe the synthesis of seven 2′-substituted MMI linked nucleosidic dimers (**1–3**), their conversions to the standard phosphoramidites, and subsequent incorporation into oligonucleotide via an automatic DNA synthesizer. The hybridization data on doubly modified oligomers is also presented which allowed the selection of a preferred 2′-substituent in combination with the MMI linkage.

## CHEMISTRY AND DISCUSSION

Our initial investigations in the 2′-deoxynucleoside series of dimeric units linked via an MMI bridge has resulted in two principal synthetic routes. First, a reductive coupling procedure[15] and second, a radical coupling[16] approach. The latter route has been published[17] covering the synthesis of various MMI linked dimers both in purine and pyrimidine bases. This methodology worked exceedingly well toward the preparation of the 2′-deoxy series of MMI dimers with complete stereo control of the 3′-α- *C*–*C* bond formation. However, when this procedure was applied to the 2′-OMe series, a nonstereoselective addition occurred (5–25% of β-isomer) with modest yields of purine containing dimers. In view of this, we chose to pursue the synthesis of 2′-modified dimers **1–3** utilizing the reductive coupling methodology (Figure 1).

We opted to synthesize dimers **1a-c** first for the following reasons. We had previously known that the top sugar residue (with 3′-*C*—*C* bond) of the 2′-deoxy MMI





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

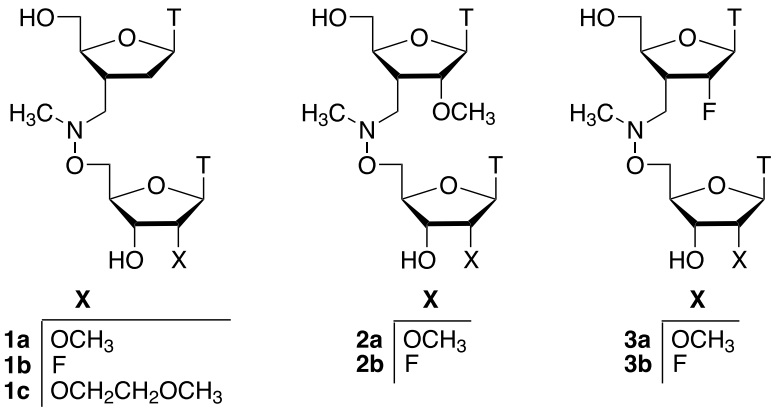


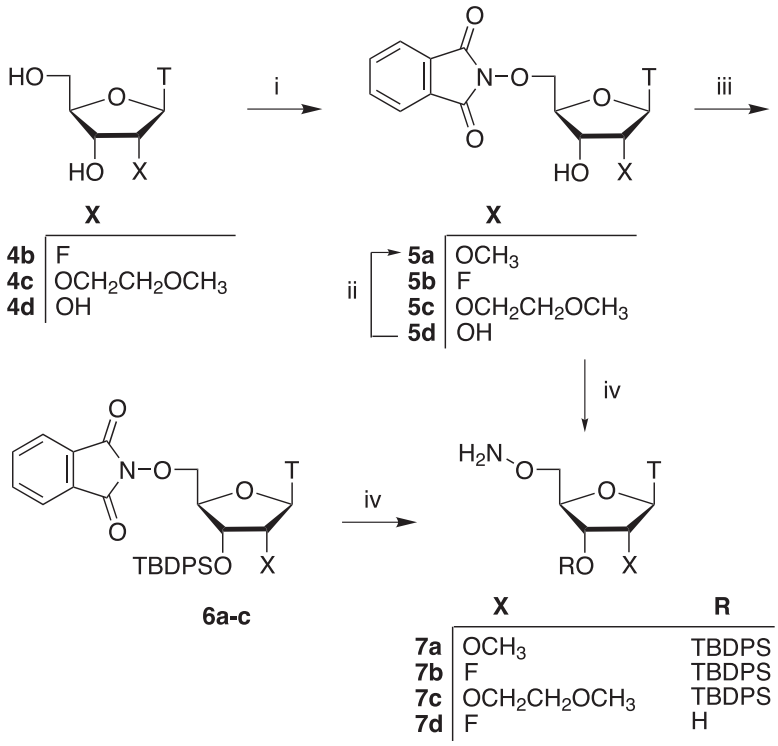

***Figure 1.*** 2′-modified MMI dimers.



dimer (**1**, x = H) exists predominantly in an RNA-like conformation (C3′-*endo*) whereas the bottom sugar residue (with 3′-*C*—*O* bond) displays frequent transitions between N ⟺ S puckered states.[18] Such unproductive motion of the sugar conformation can be reduced via a placement of a 2′-electronegative substituent in nucleosides and oligonucleotides.[19] It was therefore hoped that placement of a 2′-substituent on the lower sugar of an MMI linked dimer might drive adoption of a 3′-*endo* conformation. This would result in a uniform RNA like structure when assembled into an oligomer, and thereby increase the affinity for the RNA target. Thymine (5–methyluracil) bases were utilized as placement of a methyl group at the C5 in pyrimidines is well known to alter the hydration resulting in an enhancement of affinity for the target RNA.[20,21] Additionally, use of a thymine residue allows incorporation into oligonucleotides without base protection.

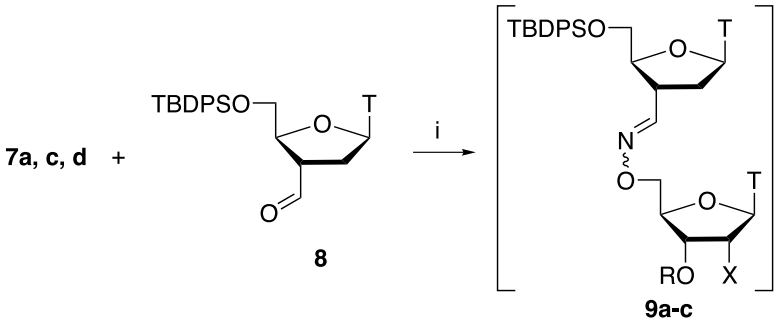
The synthesis of dimers **1a-c** was envisaged via coupling of 3′-*C*-formyl nucleoside **8** with the 5′-*O*-amino derivative **7** to provide an oxime dimer **9** which upon reduction followed by methylation and deprotection would give the desired dimer. The common top piece **8** was synthesized in a facile manner using an intermolecular radical *C*—*C* bond formation reaction reported previously.[22] The synthesis of the three-bottom pieces **7a-c** is depicted in Scheme 1. The commercial availability[a] of 5-methyluridine **4d** allowed a convenient starting-point for the synthesis of **5d**. Treatment of **4d** under Mitsunobo conditions ($HOPhth/Ph_3P/DIPAD/DMF$)[25] furnished **5d** (69%) in a regioselective manner. Subsequent methylation of **5d** using a modified protocol [$(Bu)_2SnO/(Bu)_4NI/CH_3I/DMF$)][26] gave 2′-OMe **5a** in 70% yield. However, a small amount (< 10%) of 3′-OMe isomer was also formed and removed via chromatography. The 2′-OMe **5a** was then silylated (TBDPSCl/imidazole/DMF) to give 3′-*O*-silylated **6a** (92%), which upon hydrazinolysis ($H_3CNHNH_2$) gave 5′-*O*-amino **7a** (79%) as crystalline product.

[a]Purchased from Yamasa Corporation, Summit Pharmaceuticals, 400 Kelby St., Fort Lee, NJ 07024. For a chemical synthesis see Ref. [23]. For an enzymatic synthesis see Ref. [24].




***Scheme 1.*** Reagents and Conditions: (i) *N*-hydroxyphthalimide/DEAD/$Ph_3P$/DMF; (ii) dibutyltin oxide/NaH/MeI; *t*-$BuSiPh_2Cl$/imidazole/DMF; (iv) $MeNHNH_2/CH_2Cl_2$. Abbreviations: T = thymine; TBDPS = *t*-$Bu(Ph_2)Si$.

In order to avoid the formation of minor 3′-isomer, we decided to utilize **4b**[27] and **4c**[28] as pure 2′-substituted nucleosides for the synthesis of **7b** and **7c**, respectively. Both **4b** and **4c** underwent standard Mitsunobu reaction to provide **5b** (70%) and **5c** (38%) which upon silylation, followed by hydrazinolysis of the products furnished **7b** and **7c**, respectively, in good yield. Coupling of the three aldehyde containing bottom pieces **7a-c** to the hydroxylamine **8**, was accomplished utilizing acid catalyzed coupling conditions, which were previously found to be essentially quantitative both in solution and solid-support.[29] In this manner, an equimolar mixture of **8** and **7a-c** in $CH_2Cl_2$/tolune/AcOH was coevaporated repeatedly until **8** was fully consumed (TLC) (Scheme 2). The residue was then treated independently with $NaBH_3CN$ in AcOH, followed by addition of aq. HCHO and more $NaBH_3CN$ to furnish **10a-c**, respectively, in good to excellent yields. This condensed procedure allows three reactions to be performed in one-pot (coupling/reduction/methylation), and enables the rapid construction of such dimers.[30] Desilylation (TBAF/THF) of **10a-c** furnished **1a-c**, respectively, in good yield. Diols **1a-c** were dimethoxytritylated and the products (**23a-c**) were phosphitylated in a standard manner[31] to provide **24a-c**, respectively, in good overall yield (Scheme 6).




| | X | R |
|---|---|---|
| 10a | $OCH_3$ | TBDPS |
| 10b | F | H |
| 10c | $OCH_2CH_2OCH_3$ | TBDPS |

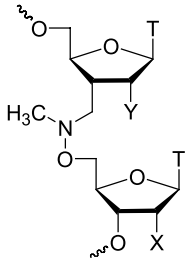
| | X |
|---|---|
| 1a | $OCH_3$ |
| 1b | F |
| 1c | $OCH_2CH_2OCH_3$ |

***Scheme 2.*** Reagents and Conditions: (i) $PhCH_3/CH_2Cl_2$/catalytic AcOH; (ii) $NaBH_3CN/CH_2O$/AcOH; (iii) TBAF/THF.

The phosphoramidites **24a-c** were then incorporated once, twice or five times into an oligomeric sequence using a standard[32] automated DNA synthesizer. The modified oligomers were HPLC purified and characterized by CGE and ES-MS (for experimental details see Refs. [33,34]). The results of the Tm studies are summarized in the Table 1. All three modifications (i.e. 2′-OMe, 2′-F, and 2′-MOE) had significant stabilizing effects in duplex formation, with a ΔTm of roughly 2°C per incorporation, when compared to MMI alone without the 2′-substituent in a heavily modified sequence (Table 1, column A). As predicted, placement of an appropriate 2′-substituent in the bottom sugar residue of an MMI dimer was indeed able to enhance the affinity of the modified oligomer for its complement RNA.

These results encouraged us to further explore the effects of 2′-modifications on the hybridization of MMI linked dimers of type **2** and **3**, in which both sugar residues have been altered. Since the effects of 2′-OMe and 2′-$OCH_2CH_2OCH_3$ substituents in the bottom residue were similar and to minimize the synthetic efforts, we chose to synthesize and study four more dimers (**2a,b** and **3a,b**) containing combinations of 2′-F and 2′-OMe substituents. We elected to employ a similar reductive coupling procedure for the synthesis of these new dimers, which requires access to nucleosides bearing a

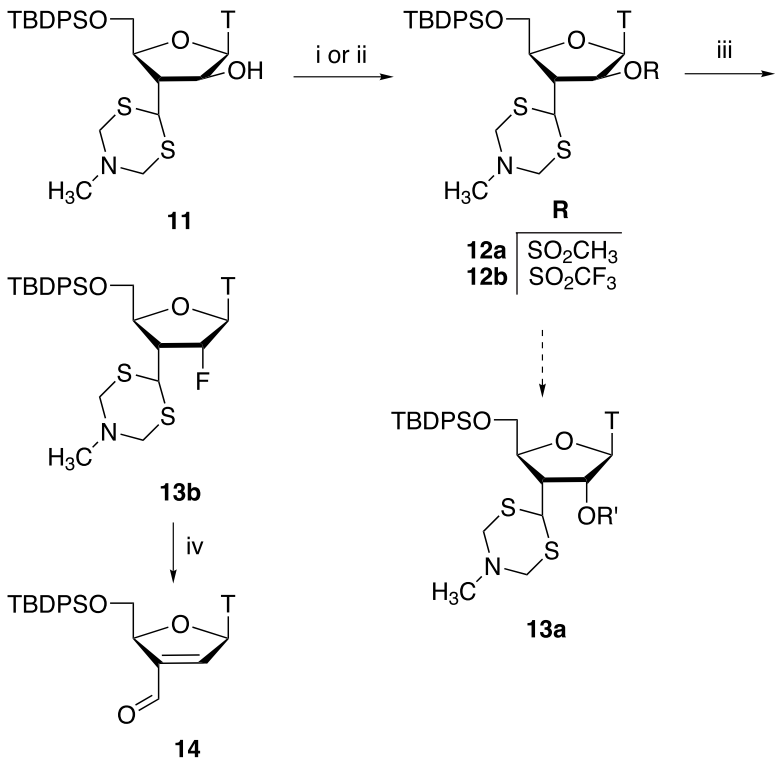
***Table 1.*** Effects of 2′-substituent on hybridization of MMI linked oligonucleotides to RNA.




| 2′-Substituent | | ΔTm per MMI dimer incorporation[b] | | | |
|---|---|---|---|---|---|
| Y (top)[a] | X (bottom)[a] | A[c] | B[c] | C[c] | Average ΔTm |
| H | H | 0.13 | − 0.23 | 1.51 | 0.47 |
| H | F | 1.83 | 1.00 | − 0.15 | 0.89 |
| H | OMe | 2.27 | 1.67 | 0.87 | 1.60 |
| H | MOE[d] | 2.13 | 1.61 | 0.95 | 1.56 |
| F | F | 3.27 | 2.20 | 1.47 | 2.31 |
| F | OMe | 3.74 | 3.10 | 1.95 | 2.93 |
| OMe | F | 3.13 | 2.50 | 1.56 | 2.39 |
| OMe | OMe | 3.71 | 2.78 | 1.85 | 2.78 |

[a]2′-substituent at the top or the bottom sugar residue(s) of the MMI dimer.
[b]ΔTm per MMI dimer incorporation is the increase in Tm of the MMI containing oligonucleotide:RNA duplex relative to an unmodified DNA:RNA duplex, normalized to the number of MMI dimers contained within the sequence. See Ref. [15] for the experimental details on measurement of Tms.
[c]Sequences of modified oligos (* = MMI linked dimer, other linkages are phosphodiester): A = GCG T*T T*T T*T T*T T*T GCG; B = CTC GTA CT*T T*TC CGG TCC; C = CTC GTA CCT*TTC CGG TCC.

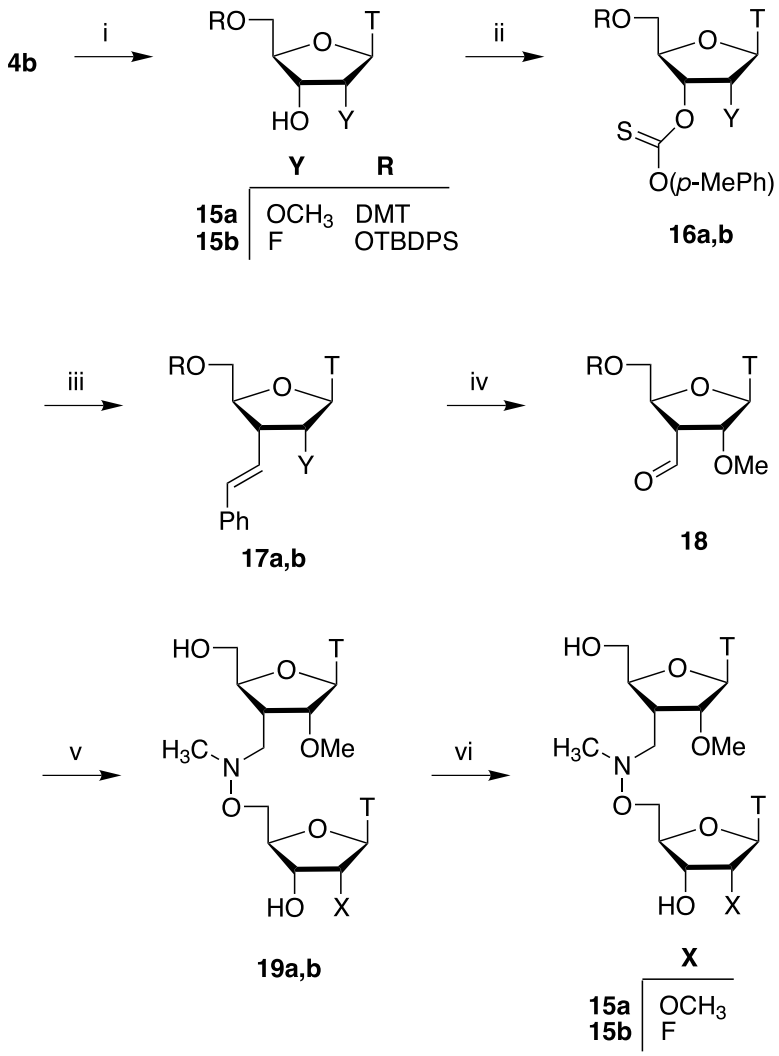
3′-*C*-formyl functionality and a 2′-F or 2′-OMe substituent. A literature search revealed that Walker and his group[35] have prepared a 3′-*C*-functionalized nucleoside **11**, which would serve as a convenient intermediate if an inversion of the 2′-hydroxyl to a 2′-F or 2′-OMe substituent could be accomplished. Scheme 3 summarizes our attempts to accomplish the intended inversion at the 2′-position of **11**. Mesylation of **11** furnished **12a** in 75% yield. Traditional methodologies[36–38] [$C_6H_5CO_2H$/CsF/DMF; $C_2H_5CO_2Cs$/DMF; $(Bu)_4N^+OH/H_2O$; NaOMe/MeOH] of inversion failed in our hands to provide **13a**. In most of these attempts mesylate **12a** was recovered unchanged. Therefore, **11** was transformed to triflate[39]**12b** (70%), which was found to be fairly stable and amenable to chromatographic purification. Again, our efforts to convert **12b** to **13a** utilizing standard conditions described above failed. Our difficulties inverting **12b** with a nucleophile continued with our attempts to prepare the 2′-fluoro substituted **13b**. Treatment of **12b** with anhydrous TBAF[38] in THF furnished **13b** and **13c** (1:1) due to concomitant deprotection of 5′-*O*-silyl group. However, silylation (TBDPSCCl/imidazole/DMF) of the mixture furnished **13b** in 70% yield. In order to unmask the 3′-




***Scheme 3.*** Reagents and Conditions: (i) $CH_3SO_2Cl$/*i*-$Pr_2NEt$/$CH_2Cl_2$; (ii) $(CF_3SO_2)_2$/pyridine/$CH_2Cl_2$; (iii) TBAF/THF; (iv) HgO/$HgCl_2$/THF/$H_2O$.

*C*-formyl group, **13b** was treated with HgO/$HgCl_2$ in wet THF at 0°C, furnishing a more polar product in 75% yield. The latter product was characterized as **14** based on $^1$H NMR, elemental analysis and HRMS. Additionally, the spectral data for **14** agreed with that of previously obtained uracil derivative reported by Walker and his group.[35] Exclusive formation of **14** from **13b** presumably occurs via mercuric salt catalyzed hydrolysis to provide the desired 3-*C*-formyl product **13d**, which subsequently eliminates HF to furnish **14**. Extensive attempts at modification of the reaction conditions to obtain **13d** were unsuccessful in our hands. The facile elimination can be attributed to the trans juxtaposition of an acidic 3′-H portion and a 2′-fluoro substituent as a good leaving group in the intermediate nucleoside **13d** during the reaction. Taken together, these unsuccessful attempts at preparation of **13a** and **13d** from **11** forced us to explore alternate routes.

One alternative strategy to gain access to 3′-*C*-formyl-2′-*O*-methyl substituted **18** is depicted in Scheme 4. To this end, we[22] have successfully demonstrated that β-tributylstannylstyrene (TBBS) mediated *C*—*C* bond formation was able to generate 3′-*C*-formyl functionality in the 2′-deoxynucleosides. Herein we now report an extension of our study to install a *C*—*C* bond in the 2′-OMe series of ribonucleosides. The synthesis of thymine analog **18** commenced with the commercial **15a**, which reacted

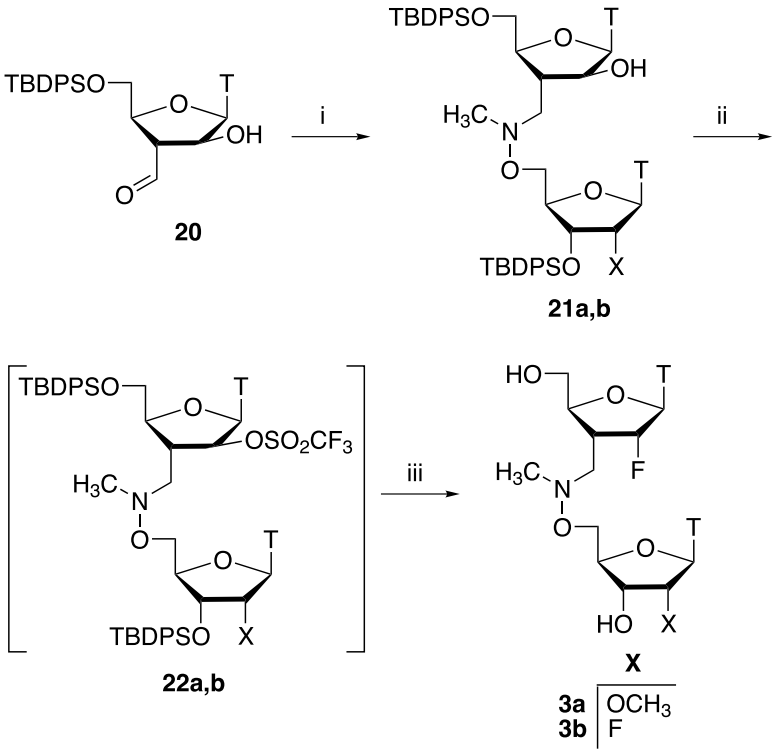



***Scheme 4.*** Reagents and Conditions: (i) *t*-BuSiPh$_2$Cl/pyridine/DMAP; (ii) *p*-MePhO(CS)Cl/DMAP/CH$_2$Cl$_2$; (iii) Bu$_3$SnCH = CHPh/AIBN/PhH/Δ; (iv) OsO$_4$/NaIO$_4$/dioxane; (v) **7a** or **7b**/PhCH$_3$/CH$_2$Cl$_2$/catalytic AcOH then NaBH$_3$CN/CH$_2$O/AcOH; (vi) TBAF/THF. Abbreviations: DMT = 4,4′-dimethoxytriphenylmethyl.

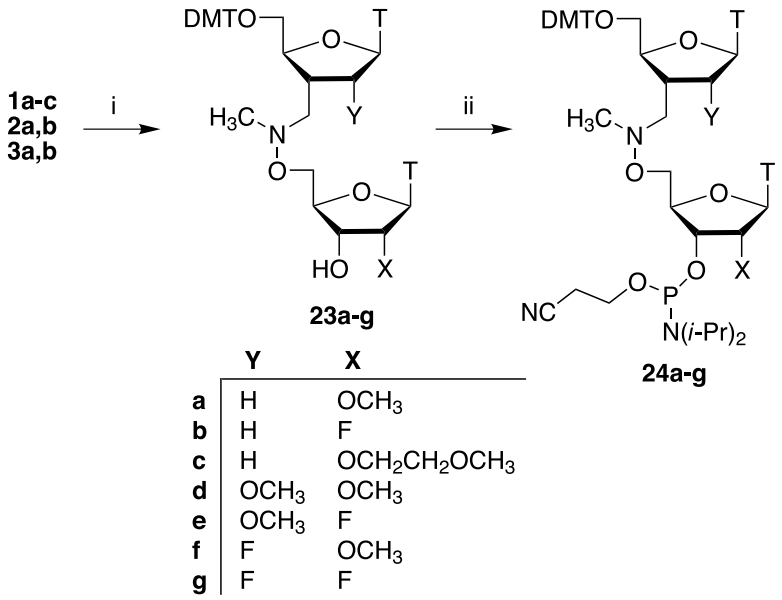
with *p*-tolyl chlorothionoformate and an excess of DMAP in CH$_3$CN to furnish **16a** (85%). Reaction of **16a** with TBBS under the conditions described earlier afforded the 3′-*C*-styryl derivative **17a** (45%) and a small amount of 3′-deoxy nucleoside (∼ 10%). The latter product was easily separated from the desired **17a** via column chromatography. Oxidative-cleavage of **17a** furnished a viable synthesis of 2′-OMe-3′-*C*-formyl nucleoside. The next consideration was the synthesis of 2′-fluoro derivative **17b** (Y = F) from the 2′-fluoro **4b** following a similar pathway. Silylation of **4b** gave

**15b** (98%), which upon thionoformylation gave **16b** (68%). Reaction of **16b** under standard radical condition furnished a complex mixture of products. The major isolated product was characterized as a mixture of two stereoisomers at the C3′-position bearing an α- and β-styrene substituent. The loss of stereoselectivity in this case is not particularly surprising because of a highly electronegative 2′-fluoro substituent that may control the outcome of radical reaction.[17]

Coupling of **18** with **7a** or **7b** under standard one-pot procedure furnished **19a** and **19b**, respectively, with concomitant deprotection of the 5′-*O*-protecting group. Desilylation of **19a** and **19b** furnished **2a** and **2b**, respectively, in good yield. Subsequently, **2a** and **2b** were transformed to the corresponding 5′-*O*-DMT-3′-*O*-amidites **24d** and **24e**, respectively, following the standard protocol in excellent yield. Various MMI modified oligonucleotides were prepared via incorporations of **24d** and **24e** and their Tms measured. Table 1 summarizes the hybridization data. The average ΔTms per modification were markedly increased via placement of a 2′-OMe substituent in the top sugar residue in combination with 2′-OMe or 2′-F substituents in the bottom sugar residues. This is likely due to affecting a C3′–*endo* conformational preorganization with bis-2′-substituted MMI modifications which facilitates binding to the complement RNA with high affinity. Additional factors such as reduced interstrand




***Scheme 5.*** Reagents and Conditions: (i) **7a** or **7b**/$PhCH_3$/$CH_2Cl_2$/catalytic AcOH then $NaBH_3CN$/$CH_2O$/AcOH; (ii) $(CF_3SO_2)_2$/pyridine/$CH_2Cl_2$; (iii) TBAF/THF.




| | Y | X |
|---|---|---|
| a | H | $OCH_3$ |
| b | H | F |
| c | H | $OCH_2CH_2OCH_3$ |
| d | $OCH_3$ | $OCH_3$ |
| e | $OCH_3$ | F |
| f | F | $OCH_3$ |
| g | F | F |

***Scheme 6.*** Reagents and Conditions: (i) DMTCl/pyridine/DMAP; (ii) 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite/diisopropylammonium tetrazolide/$CH_2Cl_2$ or 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite/$(i\text{-Pr})_2$NEt/THF.

phosphate repulsion due to neutral linkage and the 2′-electronegativity effects that stabilize the C3′-*endo* sugar pucker may contribute significantly to the affinity for the RNA target.

In order to further elaborate our SAR and understanding of the role of the 2′-electronegative substituent we forged ahead with the synthesis of **3a** and **3b** containing a 2′-fluoro substituent. Having failed twice in the direct synthesis of 2′-fluoro-3′-*C*-formyl nucleoside, we opted to take a longer but surer route. Until recently, the synthesis of 2′-fluoro modified oligonucleotides has been arduous due to lack of appropriate synthetic routes to prepare the monomeric nucleosides.[40] Although our repeated attempts to prepare monomeric building-blocks containing a 2′-fluoro-3′-*C*-formyl substituents resulted in little success, the stable 2′-fluoro-3′-*C*-(4,5-dihydro-5-methyl-1,3,5-dithiazin-2-yl) analog **13c** was easily prepared. This prompted us to undertake the synthesis of **3** via dimerization first, and followed by fluorination as shown in Scheme 5. Hydrolysis ($HgO/HgCl_2/H_2O$) of **11** furnished **20** in moderate yield. Coupling of **20** with **7a** or **7b** as described before furnished **21a** and **21b**, respectively. The subsequent fluorination of **21a** and **21b** via triflates **22a** and **22b** provided **3a** and **3b**, respectively, in good overall yield. The 3′- and 5′-silyl protecting groups were simultaneously deblocked during fluorination with TBAF in THF. The spectral ($^1$H NMR, FAB MS) data and the elemental analyses of **3** were consistent with the proposed structure. Furthermore, dimers **3** displayed a characteristic very small *J* 1′,2′ coupling constant and a large *J* 3′,4′ coupling in the $^1$H NMR spectra suggesting a high 3′-*endo* sugar conformation for both residues. Standard procedures allowed the conversions of **3a** and **3b** to the desired amidites **24f** and **24g**, respectively (Scheme 6). Incorporation of

amidites **24** into oligonucleotides was accomplished using slightly modified standard oligomerization conditions. However, due care must be taken during the base-deprotection step, which was carried out with $NH_3$/MeOH solution instead of standard $NH_4OH$ treatment in order to avoid loss of the 2′−fluoro group during deprotection.[b]

The Tm data of oligonucleotides containing dimer units **3a** and **3b** is summarized in Table 1. Once again, we observed a dramatic increase in the Tm when a 2′-fluoro substituent was placed in the top sugar residue in combination with 2′-OMe or 2′-F in the bottom units. These results are similar to that obtained with 2′-OMe modification in the top unit as in **2**. Therefore, it may be safe to assume that the contributing effects of the two very different 2′-substituents (i.e., F and OMe) is similar when used in combination with a 2′-F or 2′-OMe in the bottom sugar residue.

## CONCLUSIONS

The synthesis of seven novel nucleosidic dimers **1–3** representing a class of doubly modified (i.e., backbone and sugar) molecules useful as building-blocks for antisense constructs has been accomplished.. In the process of making these dimers, several modified nucleoside analogs (e.g., **7**, **14**, and **18**) have been prepared. Potentially, some of these may have interesting biological activity after deprotection of the blocking groups and serve as an intermediate for the synthesis of modified nucleosides of general interest (for analogs of 3′-*C*-branched nucleoside see Ref. [41]). The *one-pot* coupling/reduction/methylation procedure provides a convenient and efficient alternative to the standard multistep sequences that are commonly used to prepare functionalized amines. This procedure may also have applications in conjugation chemistry.[42]

The main focus of the study was to generate an hybridization SAR in order to assist selection of the best 2′-substitution in combination with the MMI backbone. The thermodynamic profiles obtained from this study clearly provide additional insight towards designing of high affinity antisense oligonucleotides. It has been suggested that the higher affinity of 2′-*O*-methylated RNA is entropically driven, possibly due to distortion in the minor groove hydration at the local and the global level, resulting in a net increase in the degrees of the freedom for duplex formation.[16b] Additionally, 2′-*O*-methylated RNA is conformationally preorganized due to C3′-*endo* sugar pucker that is a preferred conformation for binding to RNA. As indicated earlier, the presence of a 3′-*C*—*C* bond in the MMI linkage reduces the O4′ and C3′ gauche interaction compared to the natural phosphodiester backbone linkage. Further gain in enthalpic stabilization was realized by introducing neutral MMI linkages and stabilizing effects of a methyl group at the C5 of pyrimidine residue. The effects of the foregoing attributes were clearly visible in the Tm data generated in this study. The data unambiguously reveal that combination of an appropriate 2′-modification with a neutral and achiral linkage provides oligomers with high affinity for an RNA target.

[b]In order to release the 2′-fluoro oligomer from the support and deblock the base labile protecting groups CPG was placed in a screw-cap bottle with saturated (at 0° C) $NH_3$ in MeOH and heated at 55° C (metal block) for 12 h. For more details see reference 27.





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

From the synthesis and scale-up point-of-view, the bis–2′-OMe MMI construct has advantages which overcome the small affinity advantage of the 2′–F containing construct derived from **3a**. First, 2′-*O*-methyl-nucleosides are available from various commercial sources in kilo quantities at reasonable prices when compared to the 2′-fluoro nucleosides (particularly the purines). Second, synthetic manipulations with 2′-OMe nucleosides are much simpler and more robust compared to the 2′-F analogs, as evidenced by the difficulties encountered herein when preparing the 2′–F dimers **3a** and **3b**. Lastly, 2′-OMe analogs of antisense oligonucleotides have been recently safely evaluated in animals and in human subjects.[43] Considering both the synthetic advantages and the Tm data, we believe that the use of bis-2′-OMe substitution is the ideal 2′–modification for use in combination with MMI linkage. With high affinity for the target RNA, combined with a neutral substitution of the phosphate backbone and subsequent complete resistance to nucleolytic degradation, this construct is a promising candidate for use in future generations of antisense drugs.[44,45]



## EXPERIMENTAL SECTION

**General.** Unless otherwise noted, materials were obtained from commercial suppliers and were used as provided. Other general experimental procedures were carried out as described previously.

### General Procedures

**A. One-Pot Coupling/Reduction/Methylation.** An equimolar mixture of 5′-*O*-amino nucleoside and 3′-deoxy-3′-*C*-formyl nucleoside were dissolved in 1:1 toluene/$CH_2Cl_2$ containing several drops of acetic acid, mixed at 35°C on a rotary evaporator at atmospheric pressure for 1 h, then concentrated to a foam under reduced pressure. This process was repeated until TLC analysis (5% MeOH/$CH_2Cl_2$) showed the reaction to be complete (3–6 times), with the intermediate *E* and *Z* oximes evident as the only spot(s). The crude isomeric oxime was then dissolved in glacial acetic acid (0.1 M), placed in a cool water bath (5°C), and sodium cyanoborohydride (3 × 2 eq) was added over 0.25 h. The mixture was allowed to warm to room temperature over 1 h, placed in a cool water bath, and aqueous formaldehyde (20 eq) was added in one portion. Sodium cyanoborohydride (3 × 2 eq) was added over 0.25 h, and the mixture was allowed to warm to room temperature over 1 h. Pouring into ice water (5 times volume) with vigorous stirring terminated the reaction. The resulting residue was collected and dried, or extracted into $CH_2Cl_2$, concentrated, azeotroped (3 times) with toluene, and chromatographed. The column was eluted with 1 to 5% EtOH (95%)/EtOAc, which removed several trace impurities off the column, and the desired product was then obtained by elution with 10% MeOH/$CH_2Cl_2$, and concentration of the appropriate fractions.

**B. Desilylation.** The silylated MMI dimer was dissolved in dry THF (0.1 M) and cooled in an ice bath. A solution of tetrabutylammonium fluoride in THF (1 M, 1.5 eq per silyl group) was added dropwise. The solution was stirred at 0°C until the reaction was complete as judged by TLC (1–2 h), at which point silica (5 g/mmol) was added,



MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

and the mixture carefully concentrated. The silica was applied to a column packed in 5% MeOH/$CH_2Cl_2$, and eluted with 5% to 10% MeOH/$CH_2Cl_2$. The appropriate fractions were combined and concentrated to provide the desired dimer.

**C. Dimethoxytritylation.** The appropriate dimer (1 eq) and *N,N*-dimethylaminopyridine (0.1 eq) was azeotroped 3 times with dry pyridine, then dissolved in the minimum amount of dry pyridine. Triethylamine (2 eq) and 4,4′-dimethoxytrityl chloride were added to the stirred solution at room temperature, and the mixture diluted with dichloromethane (ca 5 mL/mL pyridine). Stirring was continued until the reaction was complete (typically overnight) as judged by TLC (10% MeOH/$CH_2Cl_2$ + 0.1% $Et_3N$), methanol was added to quench the reaction, and the reaction mixture extracted with 5% aqueous sodium bicarbonate. The organic layer was dried (trace magnesium sulfate), filtered, and concentrated to afford a syrup, which was chromatographed on silica gel ($CH_2Cl_2$ + 0.1% $Et_3N$ to 10% MeOH/$CH_2Cl_2$ + 0.1% $Et_3N$). Fractions containing only product were combined, and concentrated to afford the 5′-dimethoxytritylated compound as hard foam.

**D. Phosphitylation.** The appropriate 5′-dimethoxytrityl derivative was azeotroped with dry acetonitrile (3 ×), then dissolved in dry $CH_2Cl_2$ (0.1 M) at rt. Diisopropylammonium tetrazolide (0.5 eq) and 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (1.2 eq) was added, and the reaction allowed to stir at room temperature until complete (typically overnight) as judged by TLC (EtOAc + 0.1% $Et_3N$). The reaction mixture was then directly loaded onto a column packed with 25% EtOAc/hexane + 0.1% $Et_3N$, and eluted with a stepwise gradient to EtOAc + 0.1% $Et_3N$. Fractions containing only the product were pooled and concentrated to yield hard foam, which was lyophilized from dry 1,4-dioxane to afford the phosphoramidite as a fine white powder.

**E. Trifluoromethylsulfonylation.** A solution of dry ($P_2O_5$ overnight) hydroxyl compound (1 equiv.) in dry $CH_2Cl_2$ (25 ml/mmol) was stirred and cooled to 0–5°C. To this mixture under an inert atmosphere dry pyridine (8.5 equiv.) was added followed by dropwise addition of trifluoromethanesulfonic anhydride (1.65 equiv.) at 0–5°C. The resulting mixture was stirred for 2–4 hours. The reaction was found to be complete by TLC. In certain cases a small amount of the starting material remained, which can be further reacted with an excess of trifluoromethanesulfonic anhydride (0.1–0.2 equiv) to push the reaction to completion. At this time, the reaction mixture was poured into ice water containing saturated $NaHCO_3$ and extracted with $CH_2Cl_2$ (2 × 50 ml/mmol). In order to remove the excess of pyridine, combined organic layers were washed with cold water containing 1% AcOH (2 × 50 ml/mmol). The organic extract was dried over anhydrous $MgSO_4$ and concentrated under vacuum at room temperature to furnish the crude triflate as foam. Analytically pure sample of triflate may be obtained by silica gel column chromatography. However, in most of the examples crude triflate was carried over to the next step without purification.

**F. Fluorination/Desilylation.** Dry (coevaporated with 2 × toluene) triflate compound (1 equiv.) was dissolved in dry THF (30 ml/mmol) and cooled to 0–5°C. A solution of freshly prepared anhydrous (Ref.) TBAF in dry THF (10 ml/mmol of





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

TBAF, 3 equiv.) was added via syringe to the triflate solution at 0–5°C while stirring. After complete addition, the resulting mixture was allowed to stir for 1–2 hours. TLC indicated that reaction was usually complete at this time. Subsequently, in situ desilylation was accomplished by addition of TBAF (1.0 M in THF with ~ 5% water, 2 ml/mmol) to the reaction mixture. The reaction mixture was then stirred until a single polar spot was detected (TLC) and resulting orange colored solution was concentrated to syrup. The syrup was dissolved in $CH_2Cl_2$: MeOH (96:4, v/v) and purified by silica gel column chromatography. Elution with a mixture of $CH_2Cl_2$:MeOH furnished the desilylated fluoro compound in 70–75% yield.

**2′-*O*-Methyl-5′-*O*-phthalimido-5-methyluridine (5a).** A stirred mixture of 5′-*O*-phthalimido-5-methyluridine (40.0 g, 0.1 mol), dibutyltin oxide (29.76 g, 0.12 mol), tetrabuytylammonium iodide (40.59 g, 0.11 mol) and iodomethane (64.18 ml, 1 mol) in DMF (200 ml) was heated in a sealed flask at 50°C for 16 hours under argon atmosphere. The reaction mixture was cooled (~ 10°C) and to this another addition of methyl iodide (2.82 g, 20 mmol) was made. The flask was sealed and heating continued for 16 hours. The reaction mixture was cooled to room temperature and diluted with $CH_2Cl_2$ (50 ml). The $CH_2Cl_2$ suspension was transferred onto the top of a prepacked silica gel ($CH_2Cl_2$) column. Elution with $CH_2Cl_2$-$CH_2Cl_2$:MeOH (9:1, v/v) furnished the desired product as homogenous material. Appropriate fractions were pooled and concentrated to provide 0.6 g (69%) of the title compound (contaminated with 10% of the 3′-*O*-methyl derivative). An analytical sample was obtained by crystallization (EtOH/$CH_2Cl_2$) mp 213–214°C; $^1$H NMR (DMSO-$d_6$) 1.71 (s, 3, C$H_3$), 3.58 (s, 3, O C$H_3$), 4.42 (m, 2, 5′ C$H_2$), 4.63 (m, 1, 4′*H*), 5.36 (m, 1, 2′ *H*), 5.61 (m, 1, 3′*H*), 5.90 (s, 1, 1′*H*), 7.50 (s, 1, C6*H*), 7.82 (m, 4, Ar*H*), 11.55 (br s, t, N*H*). Anal. calc. for $C_{19}H_{19}N_3O_8 \cdot 0.5\ H_2O$: C, 53.52; H, 4.73; N, 9.85; Found: C, 53.51; H, 4.49; N, 9.84.

**5′-*O*-Phthalimido-2′-fluorothymidine (5b).** To 2′-Fluorothymidine (**4b**, 1.95 g, 7.5 mmol), triphenylphosphine (2.07 g, 7.88 mmol) and *N*-hydroxyphthalimide (1.29 g, 7.88 mmol) in dry DMF (60 mL) was added diethylazodicarboxylate (1.24 mL, 7.88 mmol) dropwise at room temperature over 0.5 h. The mixture was stirred overnight and poured into a rapidly stirred ice cold mixture of ether (150 mL) and water (150 mL). The aqueous layer was slowly diluted to a total volume of 350 mL with cold water, at which point solid began forming. The ether was decanted, an additional portion ether was added, stirred, and decanted, and the process repeated. The solution was now diluted to a total volume of 500 mL with water, and allowed to stand on ice for several hours. The solid was collected, and dried to afford 2.11 g (70%) of **5b**: $R_f$ 0.31 (5% MeOH/$CH_2Cl_2$); $^1$H NMR ($CDCl_3$) δ 8.84 (s, 1H, NH), 7.90–7.70 (m, 4H), 7.26 (s, 1H, H-6), 6.03 (dd, *J* = 3.0, 17.0 *Hz*, 1H), 5.11 (ddd, *J* = 1.5, 3.0, 52.8 *Hz*, 1H), 4.75 (m, 1H), 4.56 (m, 2H) 4.34 (m, 1H), 3.26 (br s, 1H), 1.94 (s, 3H, $CH_3$). Anal. Calcd for $C_{18}H_{16}N_3O_7F \cdot 1/4Et_2O$: C, 53.84; H, 4.40; N, 9.91. Found: C, 53.52; H, 4.60; N, 9.96.

**2′-*O*-Methoxyethyl-5′-*O*-Phthalimido-5-methyluridine (5c).** To a stirred mixture of 2′-methoxyethyl-5-methyluridine (3.16 g, 10 mmol), triphenylphosphine (2.88 g, 11 mmol) and *N*-hydroxyphthalimide (1.79 g, 11 mmol) was added dropwise diethyl





Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

azodicarboxylate (2.17 g, 12.5 mol) over a period of 1 hour at ~ 5°C. After complete addition, reaction mixture was warmed to room temperature and stirred for 16 hours. The reaction was ~ 50% (TLC, $CH_2Cl_2$:MeOH, 9:1, v/v) complete at this point of time. Therefore, another equivalent of all reagents were added in the manner described above. After second addition, the reaction mixture was stirred at room temperature for 16 hours. The reaction was ~ 80% (TLC) complete at this time, therefore, solution was concentrated under vacuum to provide a thick syrupy residue. The residue was poured into a vigorously stirred mixture of ether:ice water (100 mL:200mL) to precipitate the product. The precipitate was filtered, washed with water (3 × 100 mL) and ether (3 × 50 ml), and dried over $P_2O_5$ to provide 1.75 g (38%) of 5c: $^1$H NMR ($CDCl_3$) δ 1.98 (s, 3, $CH_3$), 3.41(s, 3, $OCH_3$), 3.52, 3.70, 3.94 and 3.99 (m, 5H), 4.06 (t, 1, 3′ O*H*), 4.32 (m, 1H), 4.51(d, 2H), 4.61 (m, 1, 4′H), 6.10 (d, 1, 1′H), 7.80 (s, 1, C6*H*), 7.86 and 7.88 (2m, 4, Ar*H*), 8.22 (brs, 1, N*H*); Anal. calc. for $C_{21}H_{23}N_3O_9$: C, 54.66; H, 5.02; N, 9.10; Found: C, 54.38; H, 5.33; N, 8.77.

**5′-*O*-Phthalimido-5-methyluridine (5d).** To a stirred mixture of 5-methyluridine (51.64 g, 0.2 mol), triphenylphosphine (57.64 g, 0.22 mol) and *N*-hydroxyphthalimide (35.86 g, 0.22 mol) was added dropwise diethyl azodicarboxylate (43.5 g, 0.25 mol) over a period of 4 hours at ~ 5°C. After complete addition, reaction mixture was warmed to room temperature and stirred for 16 hours. The reaction was ~ 50% (TLC, $CH_2Cl_2$:MeOH, 9:1, v/v) complete at this point of time. Therefore, additional quantities of triphenylphosphine (28.8 g, 0.11 mol), *N*-hydroxyphthalimide (17.9 g, 0.11 mol) and diethyl azodicarboxylate (21.7 g, 0.12 mol) were added in the manner described above. After second addition, the reaction mixture was stirred at room temperature for 16 hours. The reaction was ~ 90% (TLC) complete at this time, therefore, solution was concentrated under vacuum to provide a thick syrupy residue. The residue was poured into a vigorously stirred mixture of ether:ice water (500 mL: 1Lt) to precipitate the product. The precipitate was filtered, washed with water (3 × 100 mL) and ether (3 × 50 ml), and dried over $P_2O_5$ to provide 56 g (69%) of the title compound. An analytical sample was crystallized from EtOH:$CH_2Cl_2$, mp 236–237°C; $^1$H NMR (DMSO-$d_6$) δ 1.78 (s, 3, $CH_3$), 4.12 (m, 2′, 3′, 4′ *H*), 4.36 (m, 2, 5′$CH_2$), 5.45 and 5.62 (2d, 2, 3′, 4′ O*H*), 7.78 (s, 1, C6*H*), 7.90 (m, 4, Ar*H*), 11.32 (brs, 1, N*H*); Anal. calc. for $C_{18}H_{17}N_3O_8 \cdot 1/4\ H_2O$:C, 53.00; H, 4.32; N, 10.30; Found: C, 53.04; H, 4.28; N, 10.19.

**3′-*O*-*tert*-Butyldiphenylsilyl-2′-*O*-methyl-5′-*O*-phthalimido-5-methyluridine (6a).** A mixture of 2′-*O*-methyl-5′-*O*-phthalimido-5-methyluridine (5.5 g, 13.18 mmol), imidazole (2.68 g, 39.54 mmol) and *tert*-butyldiphenylsilylchloride (7.35 g, 26.37 mmol) in DMF (50 mL) was stirred at room temperature for 12 hours under an argon atmosphere. The reaction mixture was concentrated under vacuum to 1/4 of its volume and poured into ice water (500 ml). The aqueous mixture was extracted with $CH_2Cl_2$ (2 × 250 ml) and washed with water (2 × 100 ml) and dried ($MgSO_4$). The $CH_2Cl_2$ extract was concentrated and the residue was purified by silica gel chromatography. Elution with ether: hexanes (9:1, v/v) furnished the desired product as homogenous material. Appropriate fractions were pooled and concentrated to provide 8.0 g (92.6%) of the title compound. $^1$H NMR ($CDCl_3$) δ 1.14 (s, 9, $SiMe_3$), 1.90 (s, 3, C*H*$_3$), 3.26 (s, 3, $OCH_3$), 3.38 (m, 1, 4′*H*), 4.08 (m, 1, 2′ H), 4.23 (m, 2, 5′ C*H*$_2$), 4.50 (m, 1, 3′ *H*), 6.08 (d, 1, 1′ *H*), 7.34–7.42 (m, 10, Ar *H*), 7.60 (s, 1, C6 *H*),





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

7.65–7.85 (m, 4, Ar *H*), 8.08 (br s, 1, N*H*); Anal. Calc. for $C_{35}H_{37}N_3O_8$ Si : C, 64.10; H, 5.68; N, 6.40; Found: C, 63.96; H, 5.67; N, 6.16.

**3′-*O*-*tert*-Butyldiphenylsilyl-2′-fluoro-5′-*O*-phthalimidothymidine (6b).** Compound **5b** (6.24 g, 10 mmol) was silylated in the same manner as for **5a** to yield 5.70 g (89%) of **6b**: $R_f$ 0.76 (70% EtOAc/hexane); $^1$H NMR ($CDCl_3$) δ 8.13 (s, 1H), 7.87–7.68 (m, 4H), 7.47–7.26 (m, 10H), 6.15, (dd, J = 4.2, 14.6 Hz, 1H), 4.74 (t, 1H), 4.55 (m, 1H), 4.20 (m, 2H), 4.01 (m, 1H), 1.91 (s, 3H), 1.14 (s, 9H). Anal. Calcd for $C_{34}H_{34}N_3O_7SiF \cdot 1/2$ EtOAc : C, 62.87; H, 5.57; N, 6.11. Found: C, 62.90; H, 5.54; N, 6.25.

**3′-*O*-*tert*-Butyldiphenylsilyl-2′-*O*-methoxyethyl-5′-*O*-phthalimido-5-methyluridine (6c).** Silylation of **5c** in the same manner as for **5a** provided **6c** (84%). $^1$H NMR ($CDCl_3$) δ 1.34 (s, 9, $SiMe_3$), 1.91 (s, 3, C*H*$_3$), 3.27 (s, 3, OC*H*$_3$), 3.42, 3.63, 4.02 and 4.19 (m, 7H), 4.52 (t, 1, 3′ *H*), 6.10 (d, 1, 1′ *H*), 7.26–7.43 (m, 10, Ar *H*), 7.62 (s, 1, C6 *H*), 7.70–7.78 (m, 4, Ar *H*), 8.04 (br s, 1, N*H*).

**5′-*O*-Amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-methyl-5-methyluridine (7a).** Hydrazinolysis of **6a** in the same manner as for **7b** furnished **7a** (79%) as white foam. $^1$H NMR ($CDC1_3$) d 1.10 (s, 9, $SiMe_3$), 1.83 (s, 3, C*H*$_3$), 3.24 (m, 1, 2′*H*), 3.38 (s, 3, OC*H*$_3$), 3.63 and 3.96 (dd, 2, 5′ C*H*$_2$), 4.11 and 4.19 (2m, 2, 3′, 4′ *H*), 5.32 (br s, 2, ON*H*$_2$), 5.93 (d, 1, 1′ *H*), 7.30 (s, 1, C6*H*), 7.37–7.50 and 7.63–7.73 (m, 10, Ar *H*) and 9.22 (br s, 1, NH). Anal. Calc. for $C_{27}H_{35}N_3O_6Si \cdot 1/4\ H_2O$ : C, 61.16; H, 6.74; H, 7.92; Found: C, 61.03; H, 6.56; N, 7.86.

**5′-*O*-Amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-fluorothymidine (7b).** A solution of **6b** (5.56 g, 8.65 mmol) in $CH_2Cl_2$ (90 mL) at 0°C was treated with methylhydrazine (0.55 mL, 10.4 mmol) for 1 h with stirring, the mixture filtered, and the filtrate washed with cold $CH_2Cl_2$. The combined organics were washed with water (3 ×), dried ($MgSO_4$), diluted with toluene and concentrated, and dried to provide 4.20 g (95%) of pure **7b**: $R_f$ 0.42 (70% EtOAc/hexane); $^1$H NMR ($CDCl_3$) δ 8.39 (s, 1H), 7.68 (d, 4H), 7.45 (m, 6H), 7.15 (s, 1H), 5.92 (dd, J = 2.2, 17 Hz, 1H), 5.30 (s, 2H), 4.54 (dm, J = 53 Hz, 1H), 4.35–4.06 (m, 2H), 3.91 (dd, 1H), 3.57 (dd, 1H), 1.83 (s, 3H), 1.11 (s, 9H). Anal. Calcd for $C_{26}H_{32}N_3O_5SiF$: C, 60.80; H, 6.28; N, 8.18. Found: C, 61.01; H, 6.05; N, 8.03.

**5′-*O*-Amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-(2-methoxyethyl)-5-methyluridine (7c).** Hydrazinolysis of **6c** in the same manner as for **7b** furnished **7c** (86%) as white foam. $^1$H NMR ($CDC1_3$) d 1.11 (s, 9, $SiMe_3$), 1.82 (s, 3, C*H*$_3$), 3.24 (m, 1, 2′*H*), 3.32 (s, 3, OC*H*$_3$), 3.53, 3.58, 3.75 and 4.02 (m, 7H), 5.28 (br s, 2, ON*H*$_2$), 5.90 (d, 1, 1′ *H*), 7.31 (s, 1, C6*H*), 7.38–7.50 and 7.65–7.73 (m, 10, Ar *H*) and 7.90 (br s, 1, NH). Anal. Calc. for $C_{29}H_{39}N_3O_9Si \cdot 0.5$MeOH : C, 60.49; H, 7.06; H, 7.17; Found: C, 60.48; H, 6.96; N, 7.23.

**5′-*O*-Amino-2′-fluorothymidine (7d).** 5-*O*-Phthalimido-2′-fluorothymidine (**5b**, 4.0 g, 10 mmol) was dissolved in 100 mL of 10% MeOH/$CH_2Cl_2$ with slight warming, then cooled on ice. When the reaction mixture became cloudy, methyl-

hydrazine (0.80 mL, 15 mmol) was added dropwise. The clear reaction mixture was stirred for 1.5 h at 0°C, and the solid collected and washed with cold 10% MeOH/$CH_2Cl_2$, then dried to provide 1.99 g (72%) of **7d** (mp 165–166°C). Recrystallization from EtOH provided needles (87% recovery): mp 168–169°C; $R_f$ 0.32 (10% MeOH/$CH_2Cl_2$);$^1$H NMR (DMSO-$d_6$) δ 11.43 (s, 1H), 7.55 (s, 1H), 6.23 (s, 2H), 5.89 (dd, J = 2.0, 19.5 Hz, 1H), 5.69 (d, 1H), 5.09 (ddd, J = 2.0, 2.5, 53.3 Hz, 1H), 4.25–3.65 (m, 4H), 1.80 (s, 3H). Anal. Calcd for $C_{10}H_{14}N_3O_5F$: C, 43.64; H, 5.13; N, 15.27. Found: C, 44.00; H, 5.01; N, 15.06.

**5′-*O*-*tert*-Butyldiphenylsilyl-3′-De(oxyphosphinico)-3′-methylene(methylimino)-thymidylyl-(3′→5′)-2′-*O*-methyl-5′-*O*-*tert*-Butyldiphenylsilyl-5-methyluridine (10a).** A mixture of 5′-*O*-amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-methyl-5-methyluridine (5.25 g, 10 mmol) and 1-[5-(tert-butyldiphenylsilyl)-2,3-dideoxy-3-*C*-(formyl)-β-*D*-*erythro*-pentofuranosyl]thymine[22] (4.92 g, 10 mmol) in $CH_2Cl_2$:ACOH (50:1 mL) was stirred at room temperature for 5 minutes. The reaction mixture was then coevaporated with toluene (3 × 50 ml) under vacuum. The reaction was complete (by TLC) by the third coevaporation. The residue was dissolved in AcOH (25 ml) and cooled to ∼ 15°C. $NaCNBH_3$ (3 × 250 mg, 12 mmol) was added to the stirred reaction mixture in small portions (fume-hood). The reaction mixture was stirred for 30 min. at ∼ 15°C and to the cold solution aq. HCHO (30%, 5 ml) was added in one portion. The stirring was continued for 30 minutes and additional amount of $NaCNBH_3$ (3 × 250 mg, 12 mmol) was added in a similar manner. After 2 hours, the reaction mixture was poured into ice water (250 ml) and extracted with $CH_2Cl_2$ (2 × 250 ml). The $CH_2Cl_2$ layer was washed with water (2 × 250 ml) and dried $MgSO_4$). The solvent was removed and the residue purified by silica gel column chromatography. Elution with a gradient of $CH_2Cl_2$-$CH_2Cl_2$:MeOH (95:5, v/v) provided the desired product as homogenous material. Appropriate fractions were pooled and concentrated to furnish 5.08 g (50%) of the 3′, 5′-protected MMI dimer. $^1$H NMR ($CDCl_3$) δ 1.12 (s, 18, $SiMe_3$), 1.62 and 1.79 (2s, 6, $CH_3$), 2.45 (s, 3, N-CH3), 2.65 (m, 2, $CH_2$-N-$CH_3$), 3.30 (s, 3, O C$H_3$), 5.82 (d, 1, 1′*H*), 6.17 (t, 1, 1′*H*), 9.04 and 9.09 (2 s, 2, N*H*) and other protons.

**5′-*O*-*tert*-Butyldiphenylsilyl-3′-de(oxyphosphinico)-3′-methylene(methylimino)-thymidylyl-(3′→5′)-2′-deoxy-2′-fluoro-5-methyluridine (10b).** Reaction of 5′-*O*-amino-2′-fluorothymidine (0.54 g, 2 mmol) and 5′-*O*-*tert*-butyldiphenylsilyl-3′-deoxy-3′-*C*-formylthymidine (0.98 g, 2 mmol) according to general procedure A provided a fine, powdery solid after pouring into ice water. This material was collected, washed with water, and dried to provide 1.45 g (97%) of product that contained an impurity (ca 10%). This material was used directly in the desilylation step: $R_f$ 0.44 (10% MeOH/$CH_2Cl_2$);$^1$H NMR ($CDCl_3$) δ 9.48 (s, 1H), 9.40 (s, 1H), 7.70–7.16 (m, 12H), 6.07 (t, J = 5.7 Hz), 5.75 (d, J = 19 Hz), 5.05 (d, J = 52 Hz), 4.40–3.65 (m, 7H), 3.35 (m, 1H), 2.68 (m, 2H), 2.62 (s, 3H), 2.31 (m, 2H), 1.89 (s, 3H), 1.78 (m, 1H), 1.65 (s, 3H), 1.08 (s, 9H).

**5′-*O*-*tert*-Butyldiphenylsilyl-3′-De(oxyphosphinico)-3′-methylene(methylimino)-thymidylyl-(3′→5′)-2′-*O*-(2–methoxy)ethyl-5′-*O*-*tert*-Butyldiphenylsilyl-5-methyluridine (10c).** Reaction of 5′-*O*-amino-3′-*O*-(*tert*-butyldiphenylsilyl)- 2′-(2-metoxyethyl)thymidine (1.31 g, 2.28 mmol) and 5′-*O*-*tert*-butyldiphenylsilyl-3′-deoxy-3′-*C*-

formylthymidine (1.13 g, 2.28 mmol) according to general procedure A provided a white solid (2.12 g, 88%) after chromatography. $R_f$ 0.45 (5% MeOH/$CH_2Cl_2$); Anal. Calcd for $C_{57}H_{75}N_5O_{10}Si2 \cdot NaOAc$: C, 60.53; H, 6.74; N, 5.79. Found: C, 60.61; H, 6.67; N, 5.94.

**1-[5-*O*-(*tert*-Butyldiphenylsilyl)-3-deoxy-3-*C*-(4,5-dihydro-5-methyl-1,3,5-dithiazin-2-yl)-β-D-*arabino*-pentofuranosyl]thymine (11).** Dihydro-5-methyl-1,3,5-dithiazine (23.80 g, 176 mmol) was dissolved in dry THF (200 ml) and Hexamethylphosphoramide (HMPA) (32ml) was added. The solution was cooled to -78°C (acetone/dry ice bath) under argon atmosphere. n-Butyllithium (2.0 M in pentane, 88 ml) was then added dropwise (over 5 min.) to produce a white precipitate. Metallation was allowed to proceed for 1 hour. 1-(2,3-Epoxy-5-*O*-*tert*-Butyldiphenylsilyl-β-D-*lyxo*-pento-furanosyl)thymine (19.15 g, 40 mmol), synthesized according to the known procedure,[35] was dissolved in a mixture of dry THF (80 ml) and HMPA (108 ml). The resulting solution was added dropwise to the vigorously stirred reaction mixture, while the low temperature was maintained. After 90 min, TLC (5% MeOH/$CH_2Cl_2$) indicated no remaining starting material and a single more polar product. The reaction mixture was poured into water (400 ml), neutralized with 1M HCl and extracted with ethyl acetate. The organic phase was dried ($MgSO_4$), filtered and evaporated and the residue chromatographed on a silica gel column with 2% MeOH/$CH_2Cl_2$ to yield 16.85 g (69%) of a light yellow foam. $R_f$ 0.35 (5% MeOH/$CH_2Cl_2$); $^1$H NMR ($CDCl_3$) δ 8.61 (bs, 1H, N*H*), 7.74–7.37 (m, 11H, H-6 and $Ph_2$), 6.06 (d, 1H, J = 4.2 Hz, H-1′), 4.74–4.62 (m, 3H, H-2′ and $SCH_2N$), 4.49 (d, 1H, J = 6.4 Hz, SCHS), 4.37 (q, 1H, H-4′), 4.18–4.04 (m, 3H, H-5′ and $SCH_2S$), 4.18–4.04 (m, 3H, H-5′ and $SCH_2N$), 3.81 (dd, 1H, Ja = 2.6 Hz, Jb = 11.5 Hz, H-5′), 3.63 (d, 1H, J = 7.5 Hz, OH-2′), 2.75 (m, 1H, H-3′), 2.58 (s, 3H, N-$CH_3$), 1.69 (s, 3H, $CH_3$), 1.12 (s, 9H, *t*Bu) ppm. Anal. Calc. for $C_{27}H_{32}N_2O_6Si$ : C, 63.76; H, 6.34; N, 5.51; Found: C, 63.62; H, 6.27; N, 5.33.

**1-[5-*O*-(*tert*-Butyldiphenylsilyl)-3-deoxy-3-*C*-(4,5-dihydro-5-methyl-1,3,5-dithiazin-2-yl)-2-*O*-(trifluoromethanesulfonyl)-β-D-*arabino*-pentofuranosyl]thymine (12b).** Ara nucleoside **11** was transformed to **12b** following the general procedure E for triflate preparation in 70% yield. Anal. Calc. for $C_{31}H_{38}N_3O_7SiF_3 \cdot 2H_2O$: C, 47.62; H, 5.41; N, 5.37; Found: C, 47.34; H, 4.86; N, 5.25.

**1-[5-*O*-(*tert*-Butyldiphenylsilyl)-2,3-Dideoxy-3-*C*-(4,5-dihydro-5-methyl-1,3,5-dithiazin-2-yl)-2-fluoro-β-D-*ribo*-pentofuranosyl]thymine (13b).** Silylation of **13c** was accomplished in the same manner as for **5a** to furnish 5′-*O*-silylated **13b** (75%) as a white foam. $^1$H NMR (DMSO-$d_6$) δ 11.40 (s, 1H, NH), 7.35–7.75 (m, ArH), 5.82 (d, J = 22.0 Hz, 1′H), 5.66 and 5.41 (dd, J = 51.0 Hz and 4.0 Hz, 2′H), 2.52 (s, 3H, $NCH_3$), 1.40 (s, 3H, $CH_3$) 1.05 (s, 9H, *t*-BuH) and other sugar protons.

**1-[2,3-Dideoxy-3-*C*-(4,5-dihydro-5-methyl-1,3,5-dithiazin-2-yl)-2-fluoro-β-D-*ribo*-pentofuranosyl]thymine (13c).** Fluorination of **12b** was achieved by the general procedure F to furnish a mixture of two compounds. This mixture was further treated with TBAF to deprotect the remaining 5′-OTBPS group to furnish **13c** (73%) as a white





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

foam. $^1$H NMR (DMSO-$d_6$) δ 11.34 (s, 1H, NH), 8.01 (s, 1H, C6H), 5.86 (d, J = 18.2 Hz, 1′H), 5.48 and 5.23 (dd, J = 51.5 Hz and 4.2 Hz, 2′H), 5.39 (t, 1H, 5′OH), 4.66 and 4.33 (2m, 6H), 3.92 (m, 2H, 5′$CH_2$), 2.85 (m, 1H, 3′H), 2.50 (s, 3H, $NCH_3$), 1.76 (s, 3H, $CH_3$). Anal. Calc. for $C_{14}H_{20}N_3O_4S_2F \cdot 0.75H_2O$: C, 45.19; H, 5.99; N, 10.20; Found: C, 45.43; H, 5.72; N, 9.78.

**3′-De(oxyphosphinico)-3′-methylene(methylimino)-thymidylyl-(3′→5′)-2′-*O*-methyl-5-methyluridine (1a).** To a stirred solution of 3′, 5′-protected MMI dimer (2.2g, 1.88 mmol) in THF (25 ml) was added tetraloutylammonium fluoride (0.52 g, 2 mmol) at room temperature. The stirring was continued for 24 hours. The reaction mixture was loaded on the top of a silica gel column and elution with $CH_2Cl_2$ : MeOH (93:7, v/v). Appropriate fractions were concentrated to furnish 1.0 g (99%) of the title compound as white foam. $^1$H NMR at 60°C ($D_2O$) δ 2.26 and 2.29 (2s, 6 $CH_3$), 2.77 (m, 2, 2′ C$H_2$), 3.10 (s, 3, N-C$H_3$), 3.88 (s, 3, O-C$H_3$), 6.27 (d, 1, 1′*H*), 6.48 (t, 1, 1′ *H*), 7.90 and 8.09 (2s, 2, C6*H*) and other protons.

**3′-De(oxyphosphinico)-3′-methylene(methylimino)-thymidylyl-(3′→5′)-2′-deoxy-2′-fluoro-5-methyluridine (1b).** Crude **10b** (1.30 g, 1.70 mmol) was desilylated according to general procedure B to afford 0.65 g (67%) of **1b** as a hard foam: $R_f$ 0.29 (10% MeOH/$CH_2Cl_2$);$^1$H NMR (DMSO-$d_6$) δ 11.44 (s, 1H), 11.26 (s, 1H), 7.82 (s, 1H), (7.52 s, 1H), 6.03 (t, J = 5.3 Hz, 1H), 5.88 (dd J = 2.0, 18.9 Hz, 1H), 5.71 (d, 1H), 5.08 (t, 1H), 5.12 (dm, J = 74.4 Hz, 1H), 4.2–3.4 (m, 9H), 2.72 (m, 1H), 2.59 (s, 3H), 2.15 (m, 2H), 1.80 (s, 3H), 1.77 (s, 3H). Anal. Calcd for $C_{22}H_{30}N_5O_9F \cdot H_2O$: C, 48.44; H, 5.91; N, 12.84. Found: C, 48.80; H, 5.92; N, 12.53.

**3′-De(oxyphosphinico)-3′-methylene(methylimino)-thymidylyl-(3′→5′)-2′-*O*-(2–methoxy)ethyl-5-methyluridine (1c).** Dimer **10c** (2.02 g, 1.93 mmol) was desilylated according to the general procedure to afford 0.85 g (75%) of a white solid: $R_f$ 0.4 (10% MeOH/$CH_2Cl_2$); $^1$H NMR (DMSO-$d_6$) δ 11.35 (s, 1H), 11.23 (s, 1H), 7.82 (s, 1H), 7.53 (s, 1H), 5.99 (t, *J* = 5.0 Hz, 1H, H-1′), 5.82 (d, 1H, H-1″), 5.11 (d, 1H, 3″-OH), 5.07 (t, 1H, 5′-OH), 3.4–4.04 (m, 12H), 3.19 (s, 3H, $OCH_3$), 2.62–2.75 (m, 2H), 2.56 (s, 3H, $NCH_3$), 2.12 (m, 2H), 1.78 (s, 3H, $CH_3$), 1.75 (s, 3H, $CH_3$). Anal. Calcd for $C_{25}H_{37}N_5O_{11} \cdot 0.5H_2O$: C, 50.67; H, 6.46; N, 11.82. Found: C, 51.01; H, 6.45; N, 11.56.

**1-[5-*O*-(*tert*-Butyldiphenylsilyl)-3-deoxy-3-*C*-formyl-β-D-glycero-pent-2-eno-furanosyl]thymine (14).** Mercuric salt catalyzed oxidation of **13b** with HgO/$HgCl_2$ in wet THF at 0°C furnished the elimination product **14** in quantitative yield. $^1$H NMR (DMSO-$d_6$) δ 11.42 (s, 1H, NH), 10.01 (s, 1H, CHO), 7.00–7.65 (m, ArH), 5.18 (s, 1H, 4′H), 4.12 (m, 2H, 5′CH2), 1.11 (s, 3H, $CH_3$) 0.97 (s, 9H, *t*-BuH) and other sugar protons.

**5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(3-methylphenoxy)thiocarbonyl-2′-*O*-methyl-5-methyl-uridine (16a).** 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-methyl-5-methyluridine (14.37 g, 25 mmol) and (*N,N*-dimethylamino)pyridine (5eq) were azeotroped (3 times) in dry acetonitrile, then dissolved in acetonitrile (7 ml/mmol). A solution of *para*-toluylchlorothionoformate (4.63 ml, 30 mmol) in dry acetonitrile (20 ml) was added dropwise over 15 min. The resulting solution was stirred overnight at room





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

temperature, then quenched with few drops of water, concentrated under vacuum, extracted with $CH_2Cl_2$, washed with water, dried over sodium sulfate and finally chromatographed on silica gel column with 2% MeOH/$CH_2Cl_2$ to yield 15.55 g (86%) of the desired xanthate product as a white foam: $R_f$ 0.5 (5% MeOH/$CH_2Cl_2$); $^1$H NMR ($CDCl_3$) δ 8.20 (s, 1H, N*H*), 7.59 (s, 1H, H-6), 7.45–6.83 (m, 17H, H-Ar), 6.21 (d, 1H, J = 5.5 Hz, H-1′), 5.97 (t, 1H, J = 4.6 Hz, H-3′), 4.43 (d, 1H, J = 3.9 Hz, H-4′), 4.37 (t, 1H, J = 5.4 Hz, H-2′), 3.79 (s, 6H, O-$CH_3$), 3.55 (m, 5H, 2′-*O*-$CH_3$ , H-5′, H-5″), 2.38 (s, 3H, $CH_3$), 1.45 (s, 3H, T-$CH_3$) ppm.

**5′-*O*-*tert*-Butyldiphenylsilyl-2′–deoxy–2′–fluoro-3′-*O*-(4-methylphenoxy)thiocarbonyl -5-methyluridine (16b).** Compound **4b** (260 mg, 1 mmol) was azeotroped with dry pyridine, then dissolved in dry pyridine, and DMAP (1 mg) and *tert*-butyldiphenylsilyl chloride (280 mg, 1 mmol) were added. The solution was stirred at rt for 48 h, then concentrated, dissolved in EtOAc and washed with water (3 ×), brine, then dried ($MgSO_4$) and concentrated to yield 490 mg (98%) of crude **15b**. This material was azeotroped with $CH_3CN$ (2 ×), dissolved in $CH_2Cl_2$ (10 mL), and DMAP (171 mg, 1.4 mmol) followed *p*-tolyloxychlorothionoformate (0.19 mL, 1.2 mmol, dropwise) were added. The solution was stirred 18 h at rt, washed with water (2 ×), brine, dried ($MgSO_4$), concentrated, and chromatographed (30 to 50% EtOAc/hexane) to afford 0.44 g (68%) of **16b**: $R_f$ 0.52 (5% MeOH/$CH_2Cl_2$); $^1$H NMR ($CDCl_3$) δ 8.27 (s, 1H), 7.88 (s, 1H), 7.75–7.60 (m, 4H), 7.50–7.30 (m, 8H), 7.22 (s, 1H),7.01 (d, 2H), 6.26 (dd, J = 4.5, 15.3 Hz, 1H), 5.95 (m, 1H), 5.46 (dt, J = 4.5, 54 Hz, 1H), 4.45 (m, 1H), 4.05 (m, 1H), 2.39 (s, 3H), 2.54 (m, 1H), 1.66 (s, 3H), 1.12 (s, 9H).

**5′-*O*-dimethoxytrityl-2′-*O*-methyl-3′-deoxy-3′-*C*-styryl-5-methyluridine (17a).** A portion of this material was dissolved in MeOH/THF (1:1), and NaOMe was added to bring the pH = 10 (to wet litmus paper).[22] The mixture was stirred 24 h at rt, at which point a slightly faster moving contaminant (70% EtOAc/hexane) was converted to a much slower moving compound. The solvent was removed, the residue partitioned between EtOAc and water, and the organic layer dried ($MgSO_4$), concentrated, and chromatographed (50 to 70% EtOAc/hexane + 5 drops/L $Et_3N$) to provide pure **17a** (80–90% recovery); $R_f$ 0.66 (70% EtOAc/hexane); $^1$H NMR ($CDCl_3$) δ 8.55 (s, 1H), 8.00 (s, 1H), 7.50–7.15 (m, 14H), 6.78 (dd, 4H), 6.50 (d, J = 16 Hz, 1H), 6.17 (dd, J = 8.7, 16 Hz, 1H), 5.93 (s, 1H), 4.28 (m, 1H), 3.91 (d, 1H), 3.80–3.15 (m, 3H), 3.72 (d, 6H), 3.62 (s, 3H), 1.36 (s, 3H). Anal. Calcd for $C_{40}H_{40}N_2O_7 \cdot 0.25$ EtOAc: C, 72.12; H, 6.20; N, 4.10. Found: C, 72.15; H, 6.21; N, 4.36.

**5′-*O*-dimethoxytrityl-2′-*O*-methyl-3′-deoxy-3′-*C*-formyl-5-methyluridine (18).** To a solution of **17a** (2.1 g, 3.17 mmol) in dioxane (80 mL) was added $NaIO_4$ (2.95 g, 13.8 mmol) in water (30 mL), followed by $OsO_4$ (2% w/w in water, 1.62 mL, 0.13 mmol). The mixture was stirred at rt in the dark for 5 h, and poured into EtOAc (200 mL) and water (100 mL). The organic layer was separated, washed with water, 5% $Na_2SO_3$, water, and brine (100 mL each), then dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in the minimum amount of EtOAc, then precipitated into hexane. The solid was collected and dried to provide 1.82 g (88%) of the aldehyde, which contained 0.5 eq hexane and 1 eq water (not as hydrate of RCHO)

by $^1$HNMR and elemental analysis; $R_f$ 0.24 (5% MeOH/$CH_2Cl_2$); $^1$H NMR ($CDCl_3$) δ 9.76 (s, 1H), 7.75 (s, 1H), 7.45–7.20 (m, 9H), 6.9–6.75 (m, 4H), 5.92 (s, 1H), 4.75 (d, 1H), 4.43 (d, 1H), 3.79 (s, 6H), 3.61 (s, 3H), 3.80–3.35 (m, 3H), 1.37 (s, 3H). Anal. Calcd for $C_{18}H_{16}N_3O_7F \cdot H_2O \cdot 0.5$ hexane: C, 66.75; H, 6.69; N, 4.03. Found: C, 66.53; H, 6.75; N, 4.11.

**3′-De(oxyphosphinico)-3′-methylene(methylimino)-2′-*O*-methyl-5-methyl-uridylyl-(3′→5′)-2′-*O*-methyl-5-methyluridine (2a).** A solution of **18** (200 mg, 0.30 mmol) and **7a** (184 mg, 0.30 mmol) were coupled according to general procedure A. The crude oxime residue was treated with $Cl_3CO_2H$ (250 mg, 1.5 mmol) in $CH_2Cl_2$ (10 mL) for 10 m at rt, and then extracted with 10% $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, concentrated, and then chromatographed (70% EtOAc/hexane to 1% MeOH/EtOAc) to afford 0.17 g (71%) of the 3′-silylated oxime dimer as a mixture of E and Z isomers ($R_f$ 0.45, 10% MeOH/$CH_2Cl_2$). This material was reductively methylated as described in general procedure A to yield 160 mg (94%) of crude 3′-silylated dimer ($R_f$ 0.55, 10% MeOH/$CH_2Cl_2$) after extractive work-up. This material was desilylated according to general procedure B, then chromatographed (10% MeOH/$CH_2Cl_2$), and the residue lyophilized to provide 0.10 g (83%) of **2a**: $R_f$ 0.29 (10% MeOH/$CH_2Cl_2$); $^1$H NMR ($D_2O$) δ 7.94 (s, 1H), 7.39 (s, 1H), 5.83 (s, 1H), 5.71 (d, 1H), 4.15–3.65 (m, 9H), 3.52 (s, 3H), 3.44 (s, 3H), 3.08 (m, 1H), 2.67 (s, 3H), 2.66 (m, 1H), 2.44 (m, 1H), 1.81 (s, 3H), 1.73 (s, 3H). Anal. Calcd for $C_{24}H_{35}N_5O_{11} \cdot H_2O$: C, 49.06; H, 6.35; N, 11.92. Found: C, 49.01; H, 6.05; N, 11.55.

**3′-De(oxyphosphinico)-3′-methylene(methylimino)-2′-*O*-methyl-5-methyl-uridylyl-(3′→5′)-2′-deoxy-2′-fluoro-5-methyluridine (2b).** A solution of **18** (900 mg, 1.28 mmol) and **7b** (660 mg, 1.28 mmol) were coupled according to general procedure A. The crude oxime residue was treated with $Cl_3CO_2H$ (1.0 g,6.4 mmol) in $CH_2Cl_2$ (33 mL) for 10 m at rt, and then extracted with 10% $NaHCO_3$. The organic layer was separated, dried over $MgSO_4$, concentrated, and then chromatographed (70% EtOAc/hexane to 1% MeOH/EtOAc) to afford 0.54 g (54%) of the 3′-silylated oxime dimer as a mixture of E and Z isomers ($R_f$ 0.69 and 0.76, 1% MeOH/EtOAc). This material was reductively methylated as described in general procedure A to yield the crude 3′-silylated dimer ($R_f$ 0.50, 10% MeOH/$CH_2Cl_2$) after extractive work-up. This material was desilylated according to general procedure B, chromatographed (5–10% MeOH/$CH_2Cl_2$), and the residue lyophilized to afford 0.26 g (68%) of **2b**: $R_f$ 0.26 (10% MeOH/$CH_2Cl_2$); $^1$H NMR ($D_2O$) δ 7.93 (s, 1H), 7.38 (s, 1H), 5.85 (s, 1H), 5.78 (d, J = 20 Hz, 1H), 5.10 (dm, J = 52 Hz, 1H), 4.35–3.65 (m, 8H), 3.52 (s, 3H), 3.09 (m, 1H), 2.67 (s, 3H), 2.65 (m, 1H), 2.44 (m, 1H), 1.82 (s, 3H), 1.77 (s, 3H); HRMS (CsI/NBA) calcd for $C_{23}H_{32}N_5O_{10}F + Cs^+$ 690.1188, found 690.1199.

**1-[5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-3-*C*-formyl-β-D-*arabino*-pentofuranosyl] thymine (20).** 3′-*C*-(4,5-dihydro-5-methyl-1,3,5-dithiazin-2-yl) nucleoside (2.76 g, 4.5 mmol) was dissolved in 15% (v/v) aqueous THF (10ml/mmol), and the resulting solution was cooled to − 5°C under argon atmosphere. To this, with rapid stirring, was added red mercuric oxide (2.14 g, 2.2 eq) followed by mercuric chloride (2.69 g, 2.2 eq). Stirring was continued until appearance of a white precipitate generally after

15 min. At this time, the reaction mixture was diluted with THF (80 ml) and treated with aqueous sodium sulfide (1M, 19 ml). The black precipitate was filtered off on a pad of celite, and the filtrate was partitioned between ethyl acetate and water. Organic phases were combined, dried ($MgSO_4$), filtered, and evaporated and the residue was purified by silica gel chromatography. Elution with 3.5% MeOH/$CH_2Cl_2$ furnished the desired product as homogenous material. Appropriate fractions were pooled, concentrated, azeotroped (3 times) with dry acetonitrile to provide 1.25 g (55%) of white foam. $R_f$ 0.30 (5% MeOH/$CH_2Cl_2$); $^1$H NMR ($CDCl_3$) δ 11.35 (s, 1H, N*H*), 9.73 (d, 1H, CHO), 7.69–7.36 (m, 11H, H-6 and $Ph_2$), 6.04 (d, 1H, J = 5.8 Hz, H-1′), 5.88 (d, 1H, J = 5 Hz, OH-2′), 4.76 (q (t on $D_2O$-shake), 1H, H-2′), 4.30 (q, 1H, H-4′), 4.02–3.84 (m, 2H, H-5′ and H-5″), 3.20 (m, 1H, H-3′), 1.59 (s, 3H, $CH_3$), 1.03 (s, 9H, *t*Bu) ppm.

**5′-*O*-*tert*-Butyldiphenylsilyl-2′–arabino–3′-De(oxyphosphinico)-3′-methylene (methylimino)-5-methyluridylyl-(3′→5′)-2′-*O*-methyl-3′-*O*-*tert*-Butyldiphenylsilyl-5-methyluridine (21a).** Coupling reaction of 5′-*O*-amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-*O*-methyl-5-methyluridine (1.47 g, 2.8 mmol) and 1-[5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-3-*C*-formyl-β-D-*arabino*-pentofuranosyl] thymine (1.42 g, 2.8 mmol) according to the general procedure A provided the corresponding oxime dimer which was further reduced and methylated to yield 2.85 g (99%) of a hard foam after concentration under vacuo: $R_f$ 0.45 (5% MeOH/$CH_2Cl_2$, 2 developments); $^1$H NMR (DMSO) δ 11.39 and 11.32 (2s, 2H, N*H*), 7.68–7.35 (m, 22H, H-6 and $Ph_2$), 5.99 (d, 1H, J = 4.9 Hz, H-1′), 5.90 (d, 1H, J = 4.7 Hz, H-1″), 5.45 (d, 1H, J = 4.9 Hz, OH-2′), 4.13 (q (t on $D_2O$-shake), 1H, H-2′), 3.35 (s, 3H, O-$CH_3$), 2.42 (s, 3H, N-$CH_3$), 1.73 and 1.54 (2s, 6H, $CH_3$), 1.03 and 1.01 (2s, 18H, *t*Bu) ppm and other protons.

**5′-*O*-*tert*-Butyldiphenylsilyl-2′–arabino–3′-De(oxyphosphinico)-3′-methylene (methylimino)-5-methyluridylyl-(3′→5′)-2′-deoxy–2′–fluoro-3′-*O*-*tert*-Butyldiphenylsilyl-5-methyluridine (21b).** Coupling reaction of 5′-*O*-amino-3′-*O*-*tert*-butyldiphenylsilyl-2′-fluorothymidine (1.44 g, 2.8 mmol) and 1-[5-*O*-(*tert*-butyldiphenylsilyl)-3-deoxy-3-*C*-formyl-β-D-*arabino*-pentofuranosyl]thymine (1.42 g, 2.8 mmol) according to the general procedure A provided the corresponding oxime dimer which was further reduced and methylated to yield 2.80 g (98%) of a hard foam after concentration under vacuo.$R_f$ 0.45 (5% MeOH/$CH_2Cl_2$); $^1$H NMR (DMSO) δ 11.41 and 11.31 (2s, 2H, N*H*), 7.66–7.35 (m, 22H, H-6 and $Ph_2$), 5.98 (d, 1H, J = 5 Hz, H-1′), 5.90 (dd, 1H, $J_a$ = 2.3 Hz, $J_b$ = 18.3 Hz, H-1″), 5.43 (d, 1H, J = 4.8 Hz, OH-2′), 4.90 (dm, 1H, J = 53.7 Hz, H-2″), 2.37 (s, 3H, N-$CH_3$), 1.69 and 1.53 (2s, 6H, $CH_3$), 1.03 and 1.00 (2s, 18H, *t*Bu) ppm and other protons. Anal. Calc. for $C_{54}H_{66}N_5O_{10}Si_2F \cdot 0.5H_2O$: C, 63.01; H, 6.56; N, 6.80; Found: C, 62.97; H, 6.48; N, 6.77.

**2′–Deoxy–2′–fluoro-3′-de(oxyphosphinico)-3′-methylene(methylimino)-5-methyluridylyl-(3′→5′)-2′-*O*–methyl-5-methyluridine (3a).** Dimer **21a** (2.64 g, 2.55 mmol) was reacted with trifluoromethane sulfonic anhydride according the general procedure, except that the reaction mixture was directly chromatographed without any work-up, to provide 1.60 g (54%) of the purified triflate intermediate. This triflate dimer was fluorinated and desilylated according to the general procedures to afford 550 mg (72%) of **3a** as a white foam: $R_f$ 0.35 (10% MeOH/$CH_2Cl_2$); $^1$H NMR (DMSO) δ 11.37 (s, 2H, N*H*), 7.98 and 7.56 (2s, 2H, H-6), 5.88 (d, 1H, J = 19.1 Hz,





Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

H-1′), 5.85 (d, 1H, J = 5.1 Hz, H-1″), 5.33 (t, 1H, OH-5′), 5.27 (dd, 1H, $J_a$ = 3.6 Hz, $J_b$ = 51.9 Hz, H-2′), 5.22 (d, 1H, J = 5.8 Hz, OH-3″), 3.31 (s, 3H, O-$CH_3$), 2.62 (s, 3H, N-$CH_3$), 1.81 and 1.75 (2s, 6H, $CH_3$) ppm and other protons. MS ($FAB^+$) m/e 558 (M + H). Anal. Calc. for $C_{23}H_{32}N_5O_{10}F \cdot 0.2\ H_2O$ + 0.4 EtOH: C, 49.32; H, 6.05; N, 12.08; F, 3.28; Found: C, 49.19; H, 5.76; N, 11.71; F, 3.44.

**2′–Deoxy–2′–fluoro-3′-de(oxyphosphinico)-3′1-methylene(methylimino)-5-methyluridylyl-(3′→5′)-2′–deoxy–2′–fluoro-5-methyluridine (3b).** Dimer **21b** (2.80 g, 2.75 mmol) was reacted with trifluoromethane sulfonic anhydride according the general procedure to provide 1.45 g (48%) of the triflate intermediate. This triflate dimer was fluorinated and desilylated according to the general procedure to afford 480 mg (70%) of **3b** as a white foam: $R_f$ 0.35 (10% MeOH/$CH_2Cl_2$); $^1$H NMR (DMSO) δ 11.43 and 11.37 (2s, 2H, N*H*), 7.93 and 7.50 (2s, 2H, H-6), 5.90 (d, 1H, J = 19 Hz, H-1′), 5.87 (dd, 1H, $J_a$ = 2.0 Hz, $J_b$ = 18.5 Hz, H-1″), 5.66 (m, 1H, OH-3″), 5.30 (m, 1H, OH-5′), 5.25 (dd, 1H, $J_a$ = 3.6 Hz, $J_b$ = 51.9 Hz, H-2′), 5.06 (dm, 1H, J = 54.1 Hz, H-2″), 2.62 (s, 3H, N-$CH_3$), 1.80 and 1.74 (2s, 6H, $CH_3$) ppm and other protons. MS ($FAB^+$) m/e 546 (M + H). Anal. Calc. for $C_{22}H_{29}N_5O_9F_2 \cdot 0.6H_2O$ + 0.25EtOH: C, 47.59; H, 5.63; N, 12.33; F, 6.69; Found: C, 47.83; H, 5.26; N, 11.96; F, 6.61.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-thymidylyl-(3′→5′)-2′-deoxy-2′-fluoro-5-methyluridine (23b).** Compound **1b** (0.65 g, 1.23 mmol) was dimethoxytritylated according to general procedure C to afford 0.72 g (73%) of **23b**: $R_f$ 0.39 (10% MeOH/$CH_2Cl_2$ + 0.1% $Et_3N$);$^1$H NMR ($CDCl_3$) δ 9.45 (s, 1H), 9.42 (s, 1H), 7.71 (s, 1H), 7.50–7.10 (m, 10H), 6.82 (m, H), 6.08 (t, J = 5.4 Hz, 1H), 5.68 (d, J = 19.5 Hz, 1H), 5.07 (dd J = 54.0, 4.2 Hz, 1H), 4.31 (m, 1H), 4.15–3.75 (m, 3H), 3.78 (s, 6H), 3.58–3.18 (m, 3H), 2.68 (m, 3H), 2.60 (s, 3H), 2.38 (m, 2H), 1.80 (s, 3H), 1.49 (s, 3H).

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-thymidylyl-(3′→5′)-2′-*O*–(2–methoxy)ethyl-5-methyluridine (23c).** Compound **1c** (0.80 g, 1.36 mmol) was tritylated according to the general procedure to afford 0.80 g (66%) of the dimethoxytrityl ether **23c**: $R_f$ 0.8 (10% MeOH/$CH_2Cl_2$ + 0.1% $Et_3N$); $^1$H NMR ($CDCl_3$) δ 8.99 (brs, 2H, NH), 7.71 (s, 1H, C5H), 7.2–7.43 (m, ArH), 6.8 (m, 4H, ArH), 6.14 (t, *J* = 5.2 Hz, 1H, H-1′), 5.78 (d, 1H, H-1″), 3.78 (s, 6H, 2$OCH_3$), 3.36 (s,3H, $OCH_3$), 2.58 (s, 3H, $NCH_3$), 1.83, 1.47 (2s, 6H, $CH_3$) and other protons. MS ($FAB^+$) m/e 886 (M + H). Anal. Calcd for $C_{46}H_{55}N_5O_{13} \cdot 1.0H_2O$: C, 61.12; H, 6.36; N, 7.75. Found: C, 61.03; H, 6.20; N, 7.73.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-2′-*O*-methyl-5-methyluridine (23d).** Compound **2a** (0.30 g, 0.53 mmol) was dimethoxytritylated according to general procedure C to afford 0.36 g (79%) of **23d**: $R_f$ 0.68 (10% MeOH/$CH_2Cl_2$ + 0.1% $Et_3N$); $^1$H NMR ($CDCl_3$) δ 9.42 (s, 1H), 9.36 (s, 1H), 7.85 (s, 1H), 7.50–7.18 (m, 10H), 6.89–6.80 (m, 4H), 5.89 (s, 1H), 5.82 (d, J = 1.7 Hz, 1H), 4.25–2.50 (m, 13H), 3.79 (s, 6H), 3.60 (s, 3H), 3.58 (s, 3H), 2.58 (s, 3H), 1.88 (s, 3H), 1.36 (s, 1H). Anal. Calcd for $C_{45}H_{53}N_5O_{13} \cdot 0.5H_2O$: C, 61.35; H, 6.18; N, 7.95. Found: C, 61.43; H, 6.12; N, 7.93.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-2′-deoxy-2′-fluoro-5-methyl-uridine (23e).** Compound **2b** (0.24 g, 0.43 mmol) was dimethoxytritylated according to general procedure C to afford 0.33 g (89%) of **23e**: $R_f$ 0.46 (10% MeOH/$CH_2Cl_2$ + 0.1% $Et_3N$); $^1$H NMR ($CDCl_3$) δ 9.42 (s, 1H), 9.10 (s, 1H), 7.88 (s, 1H), 7.50–7.10 (m, 10H), 6.89–6.80 (m, 4H), 5.84 (s, 1H), 5.70 (d, J = 19 Hz, 1H), 5.07 (dd, J = 4.2, 54 Hz, 1H), 4.40–2.92 (m, 11H), 3.79 (s, 6H), 3.59 (s, 3H), 2.59 (s, 3H), 2.54 (m, 1H), 1.88 (s, 3H), 1.39 (s, 1H) ). Anal. Calcd for $C_{44}H_{50}N_5O_{12}$: C, 61.46; H, 5.86; N, 8.14. Found: C, 61.66; H, 5.89; N, 7.90.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-2′–deoxy–2′–fluoro-3′-de(oxyphosphinico)-3′-methylene(methylimino)-5-methyluridylyl-(3′→5′)-2′-*O*–methyl-5-methyluridine (23f).** Dimer **3a** (502 mg, 0.90 mmol) was tritylated following the general procedure to afford 565 mg (73%) of the 5′-dimethoxytritylated dimer **23f**: $R_f$ 0.55 (10% MeOH/$CH_2Cl_2$); $^1$H NMR (DMSO) δ 11.45 and 11.38 (2s, 2H, N*H*), 7.62 and 7.49 (2s, 2H, H-6), 7.43–6.88 (m, 13H, H-Ar), 5.89 (d, 1H, J = 20.5 Hz, H-1′), 5.81 (d, 1H, J = 5.1 Hz, H-1″), 5.37 (dd, 1H, $J_a$ = 2.6 Hz, $J_b$ = 52.6 Hz, H-2′), 5.24 (d, 1H, J = 5.8 Hz, OH-3″), 5.05 (dm, 1H, J = 55.2 Hz, H-2″), 3.73 (s, 6H, O-$CH_3$), 1.74 and 1.41 (2s, 6H, $CH_3$) ppm and other protons. Anal. Calc. for $C_{44}H_{50}N_5O_{12}F \cdot 0.3H_2O$: C, 61.07; H, 5.89; N, 8.09; Found: C, 61.00; H, 5.89; N, 7.88.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-2′–deoxy–2′–fluoro-3′-de(oxyphosphinico)-3′-methylene(methylimino)-5-methyluridylyl-(3′→5′)-2′–deoxy–2′–fluoro-5-methyluridine (23g).** Dimer **3b** (430 mg, 0.79 mmol) was tritylated following the general procedure to afford 520 mg (78%) of the 5′-dimethoxytritylated dimer **23g**: $R_f$ 0.55 (10% MeOH/$CH_2Cl_2$); $^1$H NMR (DMSO) δ 11.43 (bs, 2H, N*H*), 7.61 (s, 1H, H-6), 7.45–6.87 (m, 14H, H6 and H-Ar), 5.90 (d, 1H, J = 20.5 Hz, H-1′), 5.82 (d, 1H, J = 19.1 Hz, H-1″), 5.68 (d, 1H, J = 6.2 Hz, OH-3″), 5.35 (dd, 1H, $J_a$ = 2 Hz, $J_b$ = 52.6 Hz, H-2′), 5.05 (dm, 1H, J = 55.2 Hz, H-2″), 3.73 (s, 6H, O-$CH_3$), 2.54 (s, 3H, N-$CH_3$), 1.73 and 1.39 (2s, 6H, $CH_3$) ppm and other protons. Anal. Calc. for $C_{43}H_{47}N_5O_{11}F_2$: C, 60.91; H, 5.59; N, 8.26; Found: C, 60.84; H, 5.76; N, 8.10.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-thymidylyl-(3′→5′)-3′-*O*-(β-cyanoethyldiisopropylamino)phosphiryl-2′-*O*–methyl-5-methyluridine (24a).** Compound **1a** was treated according to general procedures C and D to provide **24a** in 92% overall yield: $^1$H NMR ($CD_3CN$) δ 1.51 and 1.75 92s, 6, C*H*$_3$), 5.85 (t, 1, 1′*H*), 6.10 (pseudo t, 1, 1′*H*) 9.17 (br s, 2, N*H*) and other protons. $^{31}$P (NMR) δ 150.9 and 151.2 ppm.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-thymidylyl-(3′→5′)-3′-*O*-(β-cyanoethyldiisopropylamino)phosphiryl-2′-deoxy-2′-fluoro-5-methyluridine (24b).** Compound **23b** (0.72 g, 0.9 mmol) was phosphitylated according to general procedure D to afford 0.79 g (85%) of **24b**: $R_f$ 0.86 (EtOAc + 0.1% $Et_3N$);$^1$H NMR ($CD_3CN$) δ 9.39 (s, 2H), 7,55–7.15 (m, 11H), 6.84 (d, 4H), 6.09 (t, J = 5.6 Hz, 1H) 5.81 (d, J = 18.8 Hz, 1H), 5.05 (dm, J = 54 Hz), 3.60 (s,





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

6H), 1.71 (s, 3H), 1.50 (s, 3H), and other protons; $^{31}$P NMR ($CD_3CN$) δ 151.66 (d, J = 9.0 Hz), 151.35 (d, J = 7.9 Hz).

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-3′-*O*-(β-cyanoethyldiisopropylamino)phosphiryl-2′-*O*-methyl-5-methyluridine (24d).** Compound **23d** (0.32 g, 0.37 mmol) was phosphitylated according to general procedure D, except 10% acetone/$CH_2Cl_2$ + 0.1% $Et_3N$ to 4% MeOH/$CH_2Cl_2$ + 0.1% $Et_3N$ was used for chromatography, affording 0.33 g (83%) of **24d**: $^1$H NMR ($CD_3CN$) δ 9.15 (bs, 2H), 7.66 (s, 1H), 7.55–7.15 (m, 10H), 6.86 (d, 4H), 5.81 (bs, 2H), 1.78 (s, 3H), 1.36 (s, 3H), and other protons; $^{31}$P NMR ($CD_3CN$) δ 151.0.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-3′-de(oxyphosphinico)-3′-methylene (methylimino)-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-3′-*O*-(β-cyanoethyldiisopropylamino)phosphiryl-2′-deoxy-2′-fluoro-5-methyluridine (24e).** Compound **23e** (0.27 g, 0.31 mmol) was phosphitylated according to general procedure D to afford 0.28 g (85%) of **24e**: $^1$H NMR ($CD_3CN$) δ 9.16 (bs, 2H), 7.66 (s, 1H), 7.55–7.15 (m, 10H), 6.86 (d, 4H), 5.80 (s, 1H), 5.76 (dd, J = 1.5, 16 Hz, 1H), 5.08 (dm, J = 55 Hz, 1H), 1.77 (s, 3H), 1.33 (s, 3H), and other protons; $^{31}$P NMR ($CD_3CN$) δ 151.8, 151.7, 151.3, 151.2.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-2′–deoxy–2′–fluoro-3′-de(oxyphosphinico)-3′-methylene(methylimino)-5-methyluridylyl-(3′→5′)-3′-*O*-(β-cyanoethyldiisopropylamino)phosphiryl-2′-*O*–methyl-5-methyluridine (24f).** Dimer **23f** (301 mg, 0.35 mmol) was phosphitylated according to general procedure D to afford 240 mg (65%) of the phosphoramidite **24f**: $R_f$ 0.55 (7% MeOH/$CH_2Cl_2$ + 0.1% $ET_3N$); $^{31}$P NMR ($CD_3CN$) δ 151.29, 151.00.

**5′-*O*-(4,4′-Dimethoxytriphenylmethyl)-2′–deoxy–2′–fluoro-3′-de(oxyphosphinico)-3′-methylene(methylimino)-5-methyluridylyl-(3′→5′)-3′-*O*-(β-cyanoethyldiisopropylamino)phosphiryl-2′–deoxy–2′–fluoro-5-methyluridine (24g).** Dimer **23g** (297 mg, 0.35 mmol) was phosphitylated according to general procedure D to afford 330 mg (89%) of the phosphoramidite **24g**: $R_f$ 0.55 (7% MeOH/$CH_2Cl_2$ + 0.1% $ET_3N$); $^{31}$P NMR ($CD_3CN$) δ 151.68, 151.48, 151.38.

**Automated incorporation of 2′-modified MMI Dimers/Assembly of oligonucleotides.** All synthesis were performed on an automated DNA synthesizer such as *Millipore Expedite* or *Applied Biosystem 380B* utilizing the standard phosphoramidite protocol.

A representative protocol:

Step 1—Detritylation: 1 μmole of 5′-DMT -3′(succinyl-CPG-$NMe_2$) nucleoside was packed into a small column and connected to a Millipore Expedite DNA synthesizer. TCA (3%) solution was pumped through the column according the standard protocol used for standard amidite chemistry.





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

Step 2—Coupling (Of the 2′,2′ Modified MMI Phosphoramidite dimers): 2′,2′ Modified MMI phosphoramidite dimers were diluted in anhydrous acetonitrile up to a concentration of 0.0673 Mol/l. Using the standard coupling step, 0.217 ml of a mixture of MMI dimer solution and 1H-tetrazole (1/1, v/v) were passed through the column with an extended coupling wait step of 300 seconds.

Step 3—*Oxidation* of the phosphite triester linkage: This step was carried out using the standard oxidizing protocol recommended for commercial deoxyribonucleoside phosphoramidites.

Step 4—*Capping*: This step was carried out using the standard capping protocol recommended for commercial deoxyribonucleoside phosphoramidites.

Step 5—*Isolation and purification*: At the end of the automated synthesis, 2′,2′ Modified MMI containing oligonucleotides were concomitantly deprotected and cleaved from the solid support by treatment with concentrated aqueous ammonia solution (30%) at 55°C for 10 hours. The ammonia solution was then evaporated and the full-length oligonucleotides will be separated from the failure sequences on reverse phase HPLC. The sequences were analyzed by HPLC, CE, and ESI–MS.



## ACKNOWLEDGMENTS

Dr. Swayze thanks Prof. Townsend for his mentorship and guidance, and is grateful for the lessons in life and science that were learned in his laboratory. We also thank Dr. Elena Lesnik and Dr. Sue Freier for melting experiments, Dr. Richard H. Griffey for stimulating discussions regarding sugar conformation, and Dr. P. Dan Cook for his support of this project.

## REFERENCES

1. Sanghvi, Y.S.; Cook, P.D. *Carbohydrate Modification in Antisense Research*; Sanghvi, Y.S., Cook, P.D., Eds.; ACS Symposium Series, American Chemical Society: Washington, DC, 1994; Vol. 580, 1–22.
2. DeMesmaeker, A.; Haner, R.; Martin, P.; Moser, H. Acc. Chem. Res. **1995**, *28*, 366–374.
3. Herdewijn, P. Liebigs Ann. **1996**, 1337–1348.
4. Varma, R.S. *Molecular Biology and Biotechnology: A Comprehensive Desk Reference*; Meyers, R.A., Ed.; VCH Publishers: NY, 1995; 617–621.
5. Sanghvi, Y.S. *Comprehensive Natural Products Chemistry*; Barton, D.H.R., Nakanishi, K., Kool, E.T., Eds.; DNA Aspects of Molecular Biology, Pergamon Press, 1999; Vol. 7, 258–311.
6. Sanghvi, Y.S.; Swayze, E.E.; Peoc'h, D.; Bhat, B.; Dimock, S. Nucleosides Nucleotides **1997**, *16*, 907–916.
7. Altmann, K.-H.; Dean, N.M.; Fabbro, D.; Freier, S.M.; Geiger, T.; Haner, R.;

Husken, D.; Martin, P.; Monia, B.P.; Muller, M.; Natt, F.; Nicklin, P.; Phillips, J.; Pieles, U.; Sasmor, H.; Moser, H.E. Chimia **1996**, *50*, 168–176.
8. Altmann, K.-H.; Fabbro, D.; Dean, N.M.; Geiger, T.; Monia, B.P.; Muller, M.; Nicklin, P. Biochem. Soc. Trans. **1996**, *24*, 630–637.
9. Damha, M.J.; Meng, B.; Wang, D.; Yannopoulos, C.G.; Just, G. Nucleic Acids Res. **1995**, *23*, 3967–3973.
10. Gryaznov, S.M.; Schultz, R.G.; Lloyd, D.H. In *XII International Roundtable Nucleosides, Nucleotides and Their Biological Applications*, La Jolla, CA, September 15–19, 1996; OP3.
11. Schultz, R.G.; Gryaznov, S.M. Nucleic Acids Res. **1996**, *24*, 2966–2973.
12. Reynolds, M.A.; Hogrefe, R.I.; Jaeger, J.A.; Schwartz, D.A.; Riley, T.A.; Marvin, W.B.; Daily, W.J.; Vaghefi, M.M.; Beck, T.A.; Knowles, S.K.; Klem, R.E.; Arnold, L.J. Nucleic Acids Res. **1996**, *24*, 4584–4591.
13. De Mesmaeker, A.; Lebreton, J.; Jouanno, C.; Fritsch, V.; Wolf, R.M.; Wendeborn, S. Synlett **1997**, *11*, 1287–1290.
14. Peoch, D.; Swayze, E.E.; Bhat, B.; Dimock, S.; Griffey, R.; Sanghvi, Y.S. Nucleosides Nucleotides **1997**, *16*, 959–962.
15. Vasseur, J.-J.; Debart, F.; Sanghvi, Y.S.; Cook, P.D. J. Am. Chem. Soc. **1992**, *114*, 4005–4006.
16. Debart, F.; Vasseur, J.-J.; Sanghvi, Y.S.; Cook, P.D. Tetrahedron Lett. **1992**, *33*, 2645–2648.
17. Bhat, B.; Swayze, E.E.; Wheeler, P.; Dimock, S.; Perbost, M.; Sanghvi, Y.S. J. Org. Chem. **1996**, *61*, 8186–8199.
18. Mohan, V.; Griffey, R.H.; Davis, D.R. Tetrahedron **1995**, *51*, 6855–6868.
19. Griffey, R.; Lesnik, E.; Freier, S.; Sanghvi, Y.S.; Teng, K.; Kawasaki, A.; Guinosso, C.; Wheeler, P.; Mohan, V.; Cook, P.D. *Carbohydrate Modification in Antisense Research*; Sanghvi, Y.S., Cook, P.D., Eds.; ACS Symposium Series, American Chemical Society: Washington, DC, 1994; Vol. 580, 212–224.
20. Sanghvi, Y.S. *Antisense Research and Applications*; Crooke, S.T., Lebleu, B., Eds.; CRC Press: Boca Raton, FL, 1993; 273–288.
21. Egli, M. Angew. Chem., Int. Ed. Engl. **1996**, *35*, 1894–1909.
22. Sanghvi, Y.S.; Bharadwaj, R.; Debart, F.; DeMesmaeker, A. Synthesis **1994**, 1163–1166.
23. Fraser, A.; Wheeler, P.; Cook, P.D.; Sanghvi, Y.S. J. Heterocycl. Chem. **1993**, *30*, 1277–1287 and references cited therein.
24. Hori, N.; Sunagawa, K.; Mikami, Y. J. Biotechnol. **1991**, *17*, 121–131.
25. Perbost, M.; Hoshiko, T.; Morvan, F.; Swayze, E.; Griffey, R.; Sanghvi, Y.S. J. Org. Chem. **1995**, *60*, 5150–5156.
26. Gopalakrishnan, V.; Kumar, V.; Ganesh, K.N. Nucleosides Nucleotides **1992**, *11*, 1263–1273.
27. Kawasaki, A.; Casper, M.; Freier, S.; Lesnik, E.; Zounes, M.; Cummins, L.; Gonzalez, C.; Cook, P.D. J. Med. Chem. **1993**, *36*, 831–838.
28. Martin, P. Helv. Chim. Acta **1995**, *78*, 486–504.
29. Morvan, F.; Sanghvi, Y.S.; Perbost, M.; Vasseur, J.-J.; Bellon, L. J. Am. Chem. Soc. **1996**, *118*, 255–256.
30. Swayze, E.E.; Sanghvi, Y.S. Synlett **1997**, 859–861.





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

31. Haly, B.; Bellon, L.; Mohan, V.; Sanghvi, Y.S. Nucleosides Nucleotides **1996**, *15*, 1383–1395 and reference cited therein.
32. Beaucage, S.L.; Caruthers, M.H. *Bioorganic Chemistry: Nucleic Acids*; Hecht, S.M., Ed.; Oxford University Press: New York, 1996; 36–74.
33. Greig, M.; Griffey, R.H. Rapid Commun. Mass Spectrom. **1995**, *9*, 97–102.
34. Srivasta, G.S.; Batt, M.; Schuette, J.; Carson, R.H.; Fitchett, J.; Lee, C.; Cole, D.J. Chromatogr., A **1994**, *680*, 469–477.
35. Bamford, M.J.; Coe, P.L.; Walker, R.T. J. Med. Chem. **1990**, *33*, 2494–2501.
36. Bell, A.A.; Lea, L.; Finn, M.; Fuente, C.; Krulle, T.M.; Davis, B.G.; Fleet, G.W. Synlett **1997**, 1077–1078.
37. Jeong, L.S.; Marquez, V.M. Chem. Lett. **1995**, 301–302.
38. Cox, D.P; Terpinski, J.; Lawrynowicz, W.J. Org. Chem. **1984**, *49*, 3216–3219.
39. *Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, 4th Edition; March, J., Ed.; John Wiley & Sons: New York, 1992; 352–357.
40. Ross, B.S.; Springer, R.H.; Sprankle, K.G.; Vasquez, G.V. Nucleosides Nucleotides **1997**, *16*, 1641–1643.
41. Mikhailopulo, A.I.; Poopeiko, N.E.; Tsvetkova, T.M.; Marochkin, A.P.; Balzarini, J.; De Clercq, E. Carbohydr. Res **1996**, *285*, 17–28 and references cited therein. For analogs of 5′-$ONH_2$ nucleoside see Ref. [25].
42. Manoharan, M. Antisense Nucleic Acid Drug Dev. **2002**, *12*, 103–128.
43. Agrawal, S.; Kandimalla, E.R. Curr. Cancer Drug Targets **2001**, *1*, 197–209.
44. Crooke, S.T. *Antisense Drug Technology Principles, Strategies and Applications*; Marcel Dekker: New York, 2001.
45. Agrawal, S.; Kandimalla, E.R. Trends Mol. Med. **2002**, *8*, 114–121.

Received August 22, 2003
Accepted October 31, 2003





MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

# Request Permission or Order Reprints Instantly!