Synthesis of Novel Nucleic Acid Mimics *via* the Stereoselective Intermolecular Radical Coupling of 3′-Iodo Nucleosides and Formaldoximes[1]

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A highly convergent free radical coupling of alkyl iodides and oximes, mediated by bis(trimethylstannyl) benzopinacolate (**8**), has been utilized to prepare a series of dimeric nucleosides as mimics of natural nucleic acids. The systematic optimization of the reaction conditions allowed for the single-step conversion of the appropriate iodides and oximes into the 2′-deoxy dimers **9** in moderate to excellent yields. For example, the reaction of 3′-deoxy-3′-iodo-5′-(triphenylmethyl)thymidine (**6a**) with 3′-*O*-(*tert*-butyldiphenylsilyl)-5′-*O*-(methyleneimino)thymidine (**7a**) in the presence of **8** in degassed benzene gave an 81% yield of 3′-de(oxyphosphinico)-3′-(methyleneimino)-5′-*O*-(triphenylmethyl)thymidylyl-(3′→5′)-3′-*O*-(*tert*-butyldiphenylsilyl)thymidine (**9a**). Similarly prepared were dimers containing both pyrimidine (thymine, 5-methylcytosine) and purine (adenine, guanine) bases. The reaction was highly stereoselective, giving only a single dimeric species having the *ribo*-configuration of the newly introduced *C*-3′-branched methylene moiety. Also prepared were dimers **16**, incorporating 2′-*O*-methyl ribonucleosides in both halves of the dimer. This required the synthesis of 3′-deoxy-3′-iodo-2′-*O*-methyl nucleosides **12** as well as 2′-*O*-methyl-5′-*O*-methyleneimino nucleosides **15**. For example, 5′-*O*-(*tert*-butyldiphenylsilyl)-3′-deoxy-3′-iodo-2′-*O*-methyl-5-methyluridine (**12e**) was prepared in 80% yield by displacement of the corresponding triflate with Bu_4NI. Also prepared were the suitably protected 3′-deoxy-3′-iodo adenosine and guanosine derivatives. Compounds **15** were prepared in high yield by a regioselective Mitsunobu reaction to give the corresponding 5′-*O*-phthalimido nucleosides **13**, which were subsequently converted to the requisite oximes **15**. In the 2′-*O*-methyl series, the pinacolate coupling reaction proceeded with efficiency equal to that observed for the 2′-deoxy series **9**, but with slightly less stereoselectivity, giving predominantly the C-3′*ribo* products **16**, contaminated with 5−25% of the epimeric material. Mixed base dimers containing both pyrimidine and purine bases at all possible positions, including purine−purine dimers were prepared. The hydroxylamine or methyleneimino (MI) backbone of several representative dimers so prepared was converted *via* methylation to give the corresponding methylenemethylimino (MMI)-linked compounds, which are novel phosphate surrogates for use in antisense oligonucleotides.

Introduction

Oligonucleotide-based antisense strategies represent a unique paradigm for the treatment of a wide variety of human diseases states. The novel utility of these agents resides in their ability to selectively prevent the expression of a particular disease-associated gene in a sequence specific manner.[4] Successful drug development based on this technology requires the synthesis and use of chemically modified oligonucleotides that render stability to nucleolytic digestion, enhance cellular uptake, and hybridize with high affinity and specificity toward the target mRNA. Ongoing synthetic studies into this broad class of compounds have focused on the chemical modification of the backbone,[5] sugar,[6] and base[7] functionalities of natural DNA (**1**) and have resulted in significant progress toward establishing oligonucleotides as viable therapeutic agents. In the arena of backbone modifications, our focus of research has been on the replacement of the natural phosphodiester linkage with a nitrogen-containing, neutral, achiral isostere. Our studies have led us to investigate the chemical and biophysical properties of several different backbones,[8]

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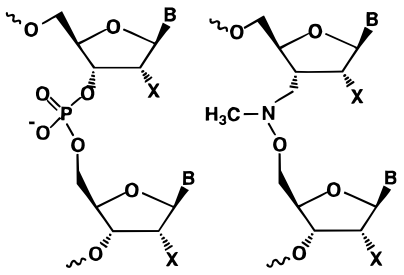
and we have selected the *m*ethylene(*m*ethyl*i*mino) (MMI, **4**) (Figure 1) linkage as a lead backbone modification for advanced studies in antisense constructs.[9]

Replacement of the phosphate backbone of an oligonucleotide with the MMI backbone has several distinct advantages in terms of its antisense properties. It provides a very high degree of nuclease resistance, the nuclease stability, as the hydroxylamine linkage will obviously not be a substrate for natural nucleases. Furthermore, we have found that an MMI linkage confers nuclease stability to the adjacent phosphodiester,[6] which allows the use of alternating phosphodiesters and MMI linkages in an antisense oligomer. The resulting alternating MMI oligomers demonstrate good affinity and specificity for complementary RNA, while reducing the net negative charge of the oligomer by more than 50%. We have also found in preliminary *in vitro* studies that MMI oligonucleotides of this type maintain or increase biological activity relative to the parent phosphorothioate oligomers.[10] Additionally, significant benefits may be realized in the economy of large-scale synthesis in solution.

In preliminary communications, we have reported two simple synthetic entries into construction of the MMI-linked thymidine dimers. The first report[8a] described an efficient coupling of 3′-C-formyl nucleoside with a 5′-*O*-amino nucleoside to furnish an oxime-linked dimer, which upon reduction, followed by in situ methylation, provided the MMI backbone. The foregoing procedure required the preparation of 3′-C-styryl nucleosides,[11] available in 30−60% yield, after labor-intensive chromatography. Such purifications become prohibitive for large-scale applications in terms of time and cost involved, and we therefore investigated alternative methods to construct the desired nucleosidic dimers. A particularly attractive and germane methodology which could be adapted for this purpose was initially reported by Hart and Seely[12] and involves the one-carbon homologation of an alkyl iodide with *O*-benzyl formaldoxime to yield an *O*-benzylhydroxylamine *via* an intermolecular, nonchain radical process. In this reaction pathway (Figure 2), bis(trimethylstannyl)benzopinacolate[13] (**8**) is decomposed thermally to provide the presumably stable stannyl ketyl radical **8a**,[14] which then can decompose to afford benzophenone and a trimethylstannyl radical.[15] Alkyl iodides react with stannyl radicals extremely rapidly[16] to irreversibly give the tin iodide, and an alkyl radical, which can then be trapped, in this case by an oxime, to provide a presumed N-centered radical species.



1-3 **4, 5**

Name	No.	B	X
DNA	**1**	A,C,G,T	H
RNA	**2**	A,C,G,U	OH
2′-OMe RNA	**3**	A,C,G,U	OCH_3
2′-Deoxy MMI	**4**	A,C,T,C^{Me}	H
2′-OMe MMI	**5**	A,C^{Me},G,T	OCH_3

Figure 1. Modified oligonucleotides.

It has been proposed by Hart et. al.[17] that this radical then combines with the stable radical **8a**, which is hydrolyzed to provide the observed *N*,*O*-dialkyl hydroxylamine. Recognizing the potential application of this methodology to a highly convergent synthesis of MMI dimers, we utilized this approach to prepare the *m*ethylene *i*mino (MI)-linked dimer **9a** (Scheme 1), although in modest 30% yield.[18] This reaction allows the installation of a key C−C bond in a chemo-, regio-, and stereoselective manner between two nucleosides, and since the hydroxylamine linkage can be readily methylated, provides an attractive approach for the synthesis of MMI dimers. The simplicity of this procedure encouraged us to further investigate the reaction in detail, with an aim to improve the coupling yield of 2′-deoxynucleosides and extend the methodology to the preparation of dimers bearing bases other than thymine.

While this study was in progress, we also became interested in the synthesis of MMI dimers modified at the 2′-position of the sugar with methoxy groups, as the stability of hybrids of 2′-*O*-Me RNA (**3**) with complementary RNA (**2**) is considerably higher ($\Delta T_m \sim 1.5$ °C/modification) than that of the corresponding DNA-RNA duplexes.[19] This increased stability has been attributed to hydrophobic interactions[20] between substituents in the minor groove and especially to the 3′-*endo* sugar conformation predominantly adopted by 2′-*O*-methyl nucleosides, which results in a decrease in the entropic motions of the sugar, while maintaining a preorganized structure with RNA-like character.[21] Additionally, chimeric antisense oligonucleotides containing **3** show enhanced stability toward nuclease digestion, higher binding affinity toward target mRNA,[22] and in some cases have shown improved properties relative to their deoxy predecessors *in vitro* and *in vivo*.[23] The potential benefits of a combination of the MMI backbone and 2′-*O*-methyl sugar modification in a single molecule, such as 2′-*O*-Me MMI

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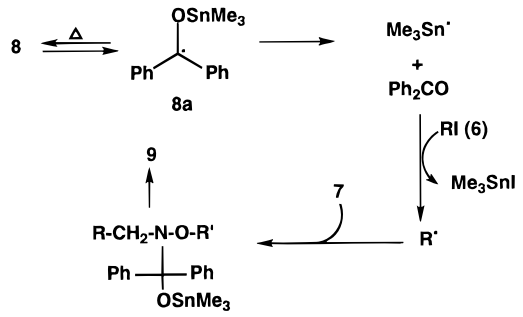

Figure 2. Pinacolate-mediated reaction pathway.

(**5**), encouraged us to extend our studies of this radical coupling reaction to the preparation of dimers of the type **5**. Herein we describe in detail a systematic synthesis study in which we have optimized the radical coupling methodology to prepare the MMI dimers of type **4**, resulting in a dramatic improvement in yield compared to our original communication. We also describe the extension of this methodology to other nucleosides, including the 2′-*O*-methyl series, resulting in the preparation of purine-containing mixed base dimers **4** and **5**. In addition, many of the requisite 3′-deoxy-3′-iodo nucleosides were unknown, and we have developed an efficient synthesis of these versatile radical precursors, as well as practical large-scale synthesis of the 5′-oxime ribonucleoside radical acceptors.

Results and Discussion

In previous studies on the synthesis of 3′-C-substituted nucleosides, we[11] and others[24] have demonstrated that control of the stereochemistry at the C3′-position of a nucleoside was possible via a radical reaction. In general, these studies have found that a C-3′ radical of a deoxy nucleoside (usually thymidine) affords mainly the 3′-*ribo* diastereomer, which results from selective attack of the radical trap from the α-face of the 3′-radical. With this consideration in mind, we envisioned the use of the *O*,*O*-bis(trimethylstannyl)benzopinacol (**8**)-mediated radical coupling reaction pathway (Figure 2) as a means to construct the desired nucleosidic dimers in a stereospecific manner from simple nucleosides in a single step. Our initial attempts at utilizing this approach focused on the preparation of a T*T deoxy MI dimer (* = 3′-CH_2NHO-5′) and required as synthons the known 3′-deoxy-3′-iodo nucleosides **6a**[25] and **6b**,[8a] as well as oxime **7a**. We have recently reported the functionalization of 2′-deoxy nucleosides to the 5′-phthalimido derivatives *via* a selective Mitsunobu reaction.[26] The oxime **7a** was readily prepared from 5′-*O*-phthalimidothymidine by silylation, cleavage of the phthalimide with methylhydrazine, and subsequent derivatization of 5′-*O*-amino-3′-*O*-(*tert*-butyldiphenylsilyl)thymidine so obtained with formaldehyde to furnish **7a** in high yield. Reaction of a 1:1:1 mixture of **6a**, **7a**, and **8** in degassed benzene resulted in the formation of a single dimeric nucleoside **9a** in 25% yield. The configuration of the dimer **9a** was determined to be 3′-*ribo*-type by 2D ^{1}H NMR techniques and was described in our initial communication of this result.[18] We believe that the stereospecifity in the formation of **9a** is due to a preferred sugar conformation, which, along with the steric effect of the 1′-nucleobase and 5′-substituent, promote a selective, unhindered α-attack on the C3′-radical center.

The proposed reaction pathway shown in Figure 2 suggests several approaches toward optimization of the yields obtained from the reaction. If the persistent radical **8a** couples with the *N*-centered radical to provide an *N*,*O*-acetal, as suggested by Hart,[17] then an increase in the amount of **8** (and therefore **8a**) present may result in more efficient trapping of the *N*-centered radical and better yields. We found that this was indeed the case, as increasing the amount of **8** to 2 and 3 equiv improved the yield from 25% to 30% and 35%, respectively (Table 1, entries 1−3). We also noticed the formation of varying amounts of the reduced product 3′-deoxy-5′-(triphenylmethyl)thymidine (**6**, X = H) in the reaction mixture, as well as traces of unreacted oxime **7a** when lower amounts of **8** were utilized. This reduced derivative presumably arises from hydrogen abstraction by the alkyl radical (from an unknown source), and the hydrogen abstraction is in direct competition with the formation of product *via* addition of the radical to **7a**. In order to assure complete utilization of the oxime, we utilized a slight excess of the iodide **6a**, along with 3 equiv of **8** (Table 1, entry 4). This resulted in complete consumption of both **6a** and **7a** and gave a 52% yield of isolated dimer **9a**, along with small amounts of 3′-deoxy nucleoside, after direct chromatography of the reaction mixture (ether, then a MeOH/CH_2Cl_2 gradient).

A number of examples in the literature describe phenyl selenides[12,27] and thionocarbonates[28] as radical precursors in carbohydrate and nucleoside chemistry. Therefore, we prepared the 3′-phenyl selenide,[29] and 3′-phenyl and 4-fluorophenyl thiocarbonate[8a] derivatives of **6** following the literature procedures. Reaction of these nucleosides (entries 5-7) under the conditions of entry 4 resulted in poor to moderate yield of the desired dimer **9b**, along with substantial amounts of unreacted starting materials and increased formation of the 3′-deoxy product **6** (R = TBDPS, X = H). We also varied the solvent to *tert*-butyl alcohol and 1,4-dioxane (entries 8, 9), which not unexpectedly decreased the yield of **9b** and increased the amount of reduced product **6** (X = H) formed.

When we employed an aqueous KF workup[30] in lieu of direct chromatography (entry 10), we obtained a marked increase in yield, isolating 81% of the dimer **9a** instead of the 52% without this treatment. This indicates the presence of an adduct which is efficiently hydrolyzed in aqueous KF to give the desired dimer in high yields and

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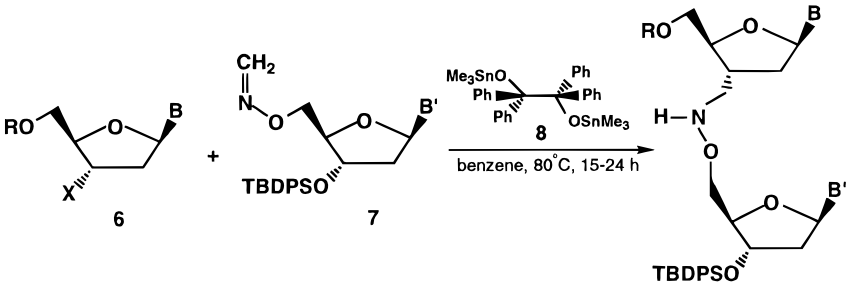
Scheme 1. Intermolecular Coupling of 2′-Deoxy Nucleosides



	B	R	X
a	T	Tr	I
b	T	TBDPS	I
c	C^{Me}	Tr	I

	B′
a	T
b	A
c	G^{dmf}

	B	B′	R
a	T	T	Tr
b	T	T	TBDPS
c	C^{Me}	T	Tr
d	T	A	Tr
e	T	G	Tr

Table 1. Optimization of Pinacolate-Mediated Nucleoside Coupling

exp no.	molar ratio[a] **6**:**7**:**8**	B	B′	R	X	solvent	% unreacted[b] **6**	% unreacted[b] **7**	% product[b] **9**
1	1:1:1	T	T	Tr	I	C6H6	0	5	25[c]
2	1:1:2	T	T	Tr	I	C6H6	0	5	30[c]
3	1:1:3	T	T	Tr	I	C6H6	0	0	35
4	1.5:1:3	T	T	Tr	I	C6H6	0	0	52[d]
5	1.5:1:3	T	T	TBDPS	SePh	C6H6	34	59	7
6	1.5:1:3	T	T	TBDPS	OC(S)OPh	C6H6	9	53	38
7	1.5:1:3	T	T	TBDPS	OC(S)OPh-F	C6H6	10	54	37
8	1.5:1:3	T	T	TBDPS	I	*t*-BuOH	27	24	35[e]
9	1.5:1:3	T	T	TBDPS	I	1,4-dioxane	19	45	22[e]
10	1.2:1:3	T	T	Tr	I	C_6H_6	0	0	81[f]
11	1:3:2	T	T	TBDPS	I	C_6H_6	0	72	84[f]

[a] All experiments were carried out on approximately 1 mmol scale. [b] Based on NMR data. [c] 5% of 2′,3′-dideoxy compound observed by NMR. [d] Isolated yield after direct chromatography of the crude reation mixture. [e] 14% of 2′,3′-dideoxy nucleoside was observed in NMR. [f] Isolated yield after aqeous KF workup and chromatography.

provides support for the pathway depicted in Figure 2. The coupling was further optimized by reducing the amount of pinacolate **8** to 2 equiv and increasing the ratio of oxime **7a** to 3 equiv, resulting in complete consumption of **6b**, total suppression of the formation of reduced product **6** (X = H, R = TBDPS), and isolation of **9b** in 84% yield (entry 11). The higher relative concentration of **7a** serves to increase the rate of addition of the alkyl radical to **7a**, thereby overwhelming the competing hydrogen abstraction pathway. The excess **7a** was easily recovered (99%, based on oxime not converted to product) and could be recycled in subsequent reactions with no reduction in yields. It should be noted that we could obtain only a modest 48% yield of **9a** by the syringe pump addition of Bu_3SnH/AIBN to a solution of **6a** (1 equiv) and **7a** (3 equiv), demonstrating the superiority of the pinacolate-mediated procedure in the synthesis dimer **9a**. These results taken together clearly indicate that use of radical precursor **6**, acceptor **7**, and pinacolate **8** in a ratio of 1:3:2, combined with an aqueous KF workup, gave the best outcome and resulted in a dramatic improvement in yield over our initial report. It is also noteworthy that the 81% of **9** (entry 10) obtained *in a single step* is far superior to the 30% obtained via our previous sequence in four steps from the radical precursor. This material can be reductively methylated to the MMI backbone in high yield and then appropriately functionalized and incorporated into oligonucleotides using standard methodology.[8a]

When we substituted the trityl-protected iodide **6a** for **6b**, otherwise using identical conditions to those in entry 11, an 81% yield of the T*T dimer **9a** was obtained, indicating there is no effect of changing the 5′-protection between Tr and TBDPS. We next extended this reaction to the preparation of mixed-base dimers. The 2′-deoxy-5-methylcytidine iodide **6c** was prepared in high yield by triazolation[31] of **6a**, followed by displacement with ammonia. Reaction of **6c** and **7a** under our optimized conditions resulted in a 58% yield of the desired ^{Me}C*T dimer **9c**, along with a 97% recovery of unreacted oxime. A preparation of 5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxy-3′-iodoadenosine has been reported utilizing Ph_3P/I_2/imidazole;[32] however, this procedure gave, in our hands, traces of the presumed 3′-iodo nucleosides and predominantly *N*-6-triphenylphosphine adducts. We made several attempts to prepare the desired 3′-iodides from base-protected purines (5′-TBDPS-dA^{Bz}, 5′-TBDPS-dA^{dmf}, and 5′-TBDPS-dG^{dmf}), using the conditions used for the synthesis of **6a** and **6b**, Ph_3P/I_2/base,[33] and various triflation/displacement conditions (*vide infra*), but were unsuccessful. Reaction of the purine 3′-thiocarbonates under the optimized conditions resulted in the formation of a ca. 40% yield of dimer, which could not be separated from ca. 20% of the corresponding 3′-deoxygenated nucleoside. We were able to demonstrate the compatibility of this reaction with purine nucleosides by the use

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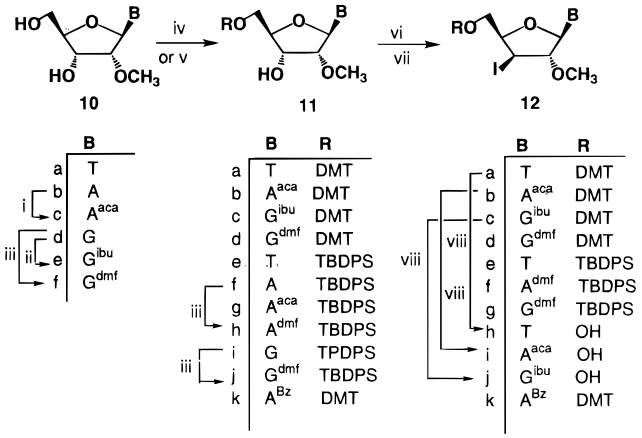
Scheme 2. Synthesis of 2′-*O*-Methyl Nucleosidic Radical Precursors[a]



[a] Reagents and conditions: (i) *N*,*N*′-dimethylacetamide dimethylacetal/MeOH; rt, 24 h; (ii) *i*BuCl/TMSCl/pyridine; rt, 12 h; (iii) *N*,*N*′-dimethylformamide diethylacetal/MeOH; rt, 24 h; (iv) DMTCl/DMAP/pyridine; rt, 16 h; (v) *t*-BuPh_2SiCl/DMAP/pyridine; rt, 15−20 h; (vi) Tf_2O/pyridine/CH_2Cl_2, −10 °C; (vii) Bu_4NI/toluene, 80 or 110 °C; (viii) 1,1,1,3,3,3-hexafluoro-2-propanol, rt, 5 h.

of oximes **7b** and **7c**. Oxime **7b** was prepared from the 5′-*O*-phthalimido derivative[26] in a manner similar to that used for **7a**. The guanosine derivative **7c** was prepared *via* a Mitsunobu reaction on *N*,*N*-dimethylformamidine (dmf) protected 2′-deoxyguanosine[34] (*vide infra*), which profoundly changes the reactivity of the guanine base.[35] Reaction of **6a** and **7b** resulted in a 59% yield of the desired T*A dimer **9d**, with recovery of 83% of unreacted **7b**. Reaction of **6a** and **7c**, however, resulted in a mixture of two dimeric and one oxime byproducts, along with unreacted **7c**. We surmised that the dmf protecting group was being partially cleaved under the reaction conditions and treated this mixture with NH_4OH/dioxane to completely remove the protecting group. This resulted in the formation of only two major products, and the base unprotected dimer **9e** was obtained in 46% yield, along with 91% of unreacted **7c**. Our ability to stereospecifically synthesize the foregoing mixed base dimers containing four types of nucleic acid bases in a single step from readily available precursors clearly demonstrates the utility of this reaction for the preparation of various heterodimers.

As discussed earlier in the introduction of this paper, due to the biological implications of 2′-*O*-methyl RNA (**3**), we concentrated our research effort on the synthesis of 2′-*O*-methyl MMI dimer **5**. This required the synthesis of the 3′-deoxy-3′-iodo nucleosides **12**. The reaction of 2′-*O*-methyl-5-methyluridine (**10a**) with 4,4′-dimethoxytrityl chloride or *tert*-butyldiphenylsilyl chloride plus a catalytic amount of DMAP in pyridine gave corresponding 5′-protected derivatives **11a** and **11e**, respectively, in excellent yields (Scheme 2). A first attempt at the iodination of **11e** was made utilizing the conditions effective for the preparation of **6b**; however, only a 27% yield of product was obtained. We next investigated several related iodination procedures, including Ph_3P/I_2/imidazole,[33a] Ph_3P/I_2/pyridine,[33b] and Ph_3P/ICH_2CH_2I/DCE[33c] without success. Reasoning that steric hindrance from the adjacent 2′-methoxy group was preventing efficient formation of the activated phosphorus species at 3′, we attempted an inversion of a 3′-triflate with iodide, which was successfully employed on carbohydrate precursors.[36] The reaction of nucleosides **11a** and **11e** with trifluoromethanesulfonic anhydride in anhydrous CH_2Cl_2 at −10 °C furnished corresponding 3′-triflates, which were stable enough to be isolated after careful aqueous workup. The crude triflates were then smoothly displaced upon heating with Bu_4NI in toluene or xylene to furnish 67% of **12a** and 80% of **12e**.

Since our studies on the pinacolate-mediated coupling reaction indicated that 3′-iodides were the ideal radical precursors for this reaction, we attempted to prepare the corresponding 3′-iodopurines *via* this sequence. 2′-*O*-Methyladenosine **10b** was protected by the reaction of nucleoside with *N*,*N*′-dimethylacetamide diethylacetal in MeOH at rt for 24 h to give corresponding acetamidine protected derivative **10c** in nearly quantitative yield, which on dimethoxytritylation afforded 70% of **11b**. For the *N*-2 protection of the guanosine analog, two separate approaches were taken. 2′-*O*-Methylguanosine (**10d**) was reacted under transient protection conditions with trimethylsilyl chloride in pyridine for 8 h, followed by addition of isobutyryl chloride to furnish the corresponding *N*-2 protected analog **10e** in 70% yield, and **10d** was reacted with *N*,*N*-dimethylformamide diethylacetal in MeOH at rt for 24 h, which furnished **10f** in 97% yield after crystallization. Subsequent tritylation of compounds **10e** and **10f** furnished corresponding 5′-protected derivatives **11c** and **11d** in 80−90% yields. As with **11a** the reaction of the purines **11b**−**d** and **11k** with trifluoromethanesulfonic anhydride furnished corresponding 3′-triflates, which were converted to 3′-iodo nucleosides **12b**−**d** and **12k** in 58−80% isolated yields upon heating with Bu_4NI in toluene or xylene. Because of problems associated with the loss of the DMT protecting group in the radical reaction and subsequent reductive methylation (*vide infra*) under acidic conditions, we also prepared the corresponding 5′-TBDPS protected purine derivatives. After silylation of **10b** and **10d** and then protection of the *N*-6 and *N*-2 positions with the dmf group to furnish **11h** and **11j**, respectively, compounds **12f** and **12g** were prepared by reaction with triflic anhydride and subsequent treatment with Bu_4NI to afford the 3′-iodo derivatives in good yield.

The structures of 3′-iodonucleosides **12** were confirmed by a broad array of spectroscopic and analytical data including ^{1}H NMR, ^{13}C NMR, FAB MS, and elemental analysis. In the ^{1}H NMR spectra, a distinct downfield chemical shift of the H3′ was observed by about 0.4 to 0.5 ppm from its precursors, which suggested the presence of an electron-withdrawing group at the 3′-position. In the ^{13}C NMR spectra, the C3′ resonance appears at ca. 26 ppm, which is indicative of the substitution of iodine for hydroxyl, as the "heavy atom effect" of iodine replaces the purely electronegative effect of oxygen.[37] The nucleophilic displacement of 3′-triflates with iodide is expected to proceed with total inversion of configuration giving the *xylo*-3′-iodo nucleoside derivatives. In order to unequivocally prove this stereochemistry by 2D NMR spectroscopy, compounds **12a**−**c** were detritylated under

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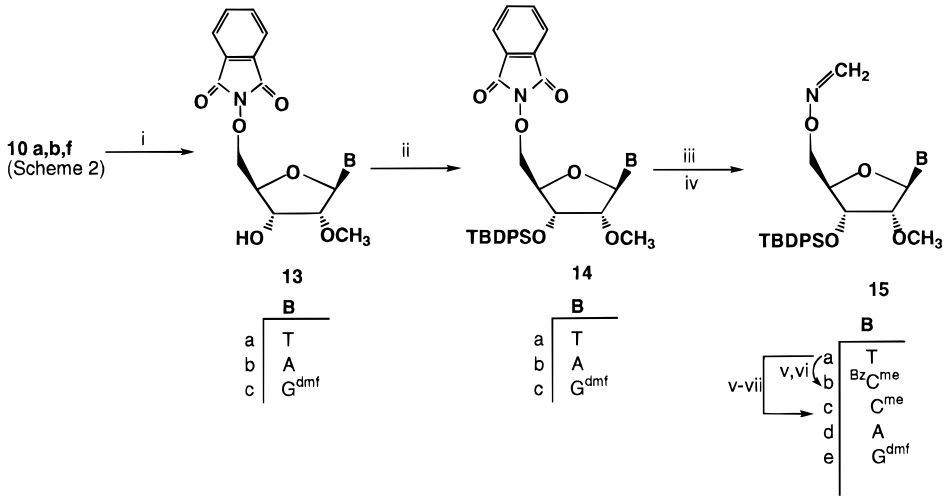
(35) A thorough investigation of this potentially useful observation is ongoing, and detailed results will be reported in due course.

(36) Binkley, R. W.; Ambrose, M. G.; Hehemann, D. G. *J. Org. Chem.* **1980**, *45*, 4387−4391.

(37) Silverstein, R. M.; Bassler, G. C.; Morrill, T. C. *Spectrometric Identification of Organic Compounds*; John Wiley & Sons: New York, 1981; p 267.

Scheme 3. Synthesis of 2′-*O*-Methyl Nucleosidic Radical Acceptors[a]



[a] Reagents and conditions: (i) Ph_3P/DEAD/PhthNOH/THF or DMF, rt, 1−3 h; (ii) *t*-$BuPh_2SiCl$/imidazole/DMF, rt, 24 h; (iii) H_3CNHNH_2/CH_2Cl_2, −10 °C−rt, 0.5−1 h; (iv) HCHO,MeOH/EtOAc, 10 °C−rt, 2−4 h; (v) 1,2,4-triazole/$POCl_3$/Et_3N/CH_3CN; (vi) NaH/benzamide/dioxane; (vii) NH_3/dioxane, rt, 1−2 h.

mild conditions using hexafluoro-2-propanol[38] at room temperature for 1−4 h to furnish corresponding 5′-hydroxy derivatives **12h**−**j** in 75−85% yield. The samples were studied in DMSO-d_6, and in each case, two-dimensional TOCSY data was used to assign the ^{1}H spectrum, and a two-dimensional NOESY was used to confirm the stereochemistry. The 3′ proton of each compound was inferred to be on the α-face of the sugar through the lack of any observable NOE between the 3′ proton and H6 of the pyrimidine or H8 of the purine. A strong NOE was evident between the 2′ sugar proton and the H6 or H8, as well as a weak NOE between the 1′ proton and H6 or H8. A weak NOE between H3′ and H1′ of each compound provided unequivocal proof of the *xylo* configuration of the 3′-iodide. Specifically, for **12h**, there were strong NOE's from H6 to H2′ and the thymine methyl, from H3′ to H4′ and between H5′ and H5″. Weak NOE's were observable between H6 and H1′, and from H1′ to H2′, H3′, H4′, and the 2′ methoxy. For **12i**, moderate NOE was observed from H1′ to H8, H2′, H3′, H4′, and the 2′ methoxy group, and from H3′ to H2′, H4′, and the 2′ methoxy group. For **12j**, strong NOE was observed between H8 and H2′, and between H3′ and H4′. Moderate NOE was observed from H1′ to H2′, H4′, and the 2′ methoxy, and a weak NOE was evident between the H1′ and H3′. We believe that we are providing the first reliable method to synthesize 2′-*O*-methyl-3′-iodo nucleosides in modest to very high yields.

The synthesis of the 2′-*O*-methyl nucleoside formaldoximes as the radical acceptors (Scheme 3) was carried out *via* a regiospecific Mitsunobu reaction of the primary 5′-hydroxyl group with *N*-hydroxyphthalimide,[39] in a manner similar to that previously described by us for the preparation of 5′-*O*-amino-2′-deoxy nucleosides.[26] Reaction of **10a** with *N*-hydroxyphthalimide in presence of triphenylphosphine and diethyl azodicarboxylate (DEAD) in THF gave a 69% yield the corresponding 2′-*O*-methyl-5′-phthalimido derivative **13a**, which crystallized directly from the reaction mixture. Reaction of **10b** under similar conditions in DMF provided the 2′-*O*-methyladenosine analog in 70% yield, after precipitation of the product from a heterogeneous ether/water mixture. We[26] and others[40] have encountered difficulty in achieving a selective Mitsunobu reaction at the 5′-hydroxyl of guanosine derivatives, as *N*-3,5′-cyclonucleosides are formed, with intramolecular attack of the *N*-3 lone pair at the activated 5′-carbon being preferred to intermolecular attack by the desired nucleophile. *O*-6 Alkylation has also been observed under Mitsunobu conditions, which further complicates the synthesis of 5′-substituted guanosine derivatives *via* a Mitsunobu reaction.[41] In the course of investigating various protecting groups for the *N*-2 of the guanine nucleobase, we observed that *N*-2 dmf protection completely suppresses undesirable side reactions at *N*-3 and *O*-6 and gives exclusively the desired 5′-substitution reaction. In this manner, 2′-*O*-methylguanosine (**10f**) was reacted with *N*-hydroxyphthalimide in presence of Ph_3P and DEAD in dry DMF, to afford a 93% yield of **14c**, which crystallized directly from the reaction mixture. The structure was confirmed by its ^{1}H NMR, in which the NH signal in anhydrous DMSO-d_6 appeared as a sharp singlet at 11.37 ppm, exactly at the same place as in **10f**. The chemical shifts of formamidine proton and H-8 also remained the same. However, a downfield chemical shift was observed for 4′ and 5′ protons by about 1.0 ppm, suggesting that the phthalimido group was attached at the 5′-position because of the magnetic anisotropic effect[42] of two amide carbonyl groups of the phthalimido group onto the 4′ and 5′ protons. The regioselectivity of the Mitsunobu reaction on **10f** is, we believe, due to the change in the electronics of the nucleobase induced by the dmf moiety at the *N*-2 position.[35]

The 5′-phthalimido derivatives **13a**−**c** were then treated with *tert*-butyldiphenylsilyl chloride and imidazole in anhydrous DMF for 24−48 h to give corresponding 3′-silyl derivatives **14a**−**c** in 90−95% yield. In general we

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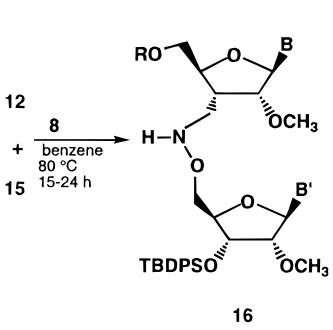
(39) (a) Mitsunobu, O. *Synthesis* **1981**, 1−28. (b) Hughes, D. in *Organic Reactions*; L. A. Paquette et. al., Eds.; John Wiley & Sons: New York, 1992; Vol. 42, pp 355−656.
(40) (a) Gao, X.; Gaffney, B. L.; Hadden, S.; Jones, R. A. *J. Org. Chem.* **1986**, *51*, 755. (b) Kimura, J.; Fujisawa, Y.; Yoshizawa, T.; Fukuda, K.; Mitsunobu, O. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1191−1196.
(41) Himmelsbach, F.; Schulz, B. S.; Trichtinger, T.; Charubala, R.; Pfleiderer, W. *Tetrahedron*, **1984**, *40*, 59−72.
(42) Vul'fson, S. G.; Nikolaev, V. F.; Vereshehagin, A. N. *Bull. Acad. Sci. USSR Div. Chem. Sci.* **1985**, *34*, 732.

Scheme 4. Intermolecular Radical Coupling of 2′-*O*-Methyl Nucleosides



	R	B	B'	%yield
a	DMT	T	T	70
b	DMT	T	C^{Me}	75
c	DMT	A^{aca}	T	52
d	DMT	G^{ibu}	T	40
e	DMT	G^{ibu}	C^{Me}	55
f	DMT	G^{ibu}	A	30
g	DMT	A^{aca}	A	40
h	TBDPS	T	T	84
i	TBDPS	A^{dmf}	A	30
j	TBDPS	A^{dmf}	G^{dmf}	40
k	TBDPS	G^{dmf}	G^{dmf}	35
l	OH	T	T	68

utilized the 3′-*O*-silyl derivatives after organic−aqueous workup directly for further transformation because silyl byproducts were the only impurities present and did not interfere in the subsequent hydrazinolysis. Deprotection of phthalimido derivatives **14a**−**c** with methylhydrazine gave corresponding intermediate nucleosides, which were found to be somewhat unstable if left unused for more than several days, even if stored in the freezer. However, the structure of each of these 5′-*O*-amino intermediates was confirmed by ^{1}H NMR and FAB MS. Because of their unstable character, the 5′-*O*-amino nucleosides were immediately reacted with aqueous formaldehyde in MeOH/EtOAc to provide the corresponding formaldoxime derivatives **15a**, **15d**, and **15e** in 70−80% overall yields from the unsubstituted nucleosides **10a**,**b**,**f**. The structures were confirmed by ^{1}H NMR, which indicated the complete disappearance of the hydroxylamine proton signal, and the appearance of the methylene protons as two distinct doublets, each showing geminal coupling with J values of 7−8 Hz. As it has been demonstrated that presence of a 5-methyl group on the cytosine residue improved the antisense properties of oligonucleotides compared to unsubstituted cytidine,[43] the 5-methylcytidine derivatives **15b** and **15c** were prepared *via* triazolation of **18a** according to the literature procedure,[28] followed by either displacement with sodium benzamide[44] to give **15b**, or with anhydrous ammonia in dioxane to afford **15c** in excellent yields.

With a full complement of 2′-*O*-methyl radical precursors **12** and acceptors **15** in hand, we began a detailed study of the ability of these synthons to be coupled into nucleosidic dimers (Scheme 4). The coupling of iodide **12a** (1 equiv) and oxime **15a** (3.0 equiv) in the presence of **8** (2 equiv), followed by an aqueous KF workup and chromatography, afforded a 50% yield of the dimer **16a**, along with unreacted oxime (90%, based on amount not converted to product). We observed several minor DMT-containing (by TLC charring) side products in this reaction, presumably arising from less efficient addition to the oxime **15a**, allowing the incipient 3′-radical to react *via* undesired pathways. Hart has observed side reactions involving various adducts with **8a**,[17] and in order to minimize this potential problem, we lowered the effective concentration of **8a** *via* the syringe pump addition of **8** to a mixture of **12a** and **15a** over 24 h. This procedure succeeded in reducing the incidence of side products and increased the yield of **16a** to 70%, along with a 90% recovery of unreacted oxime.

The reaction of benzoyl protected iodide **12k** with benzoyl protected oxime **15b** in an attempt to obtain a fully base protected A*C^{Me} dimer, gave an intractable mixture of products. Reaction of **12a** with the benzoyl protected oxime **15b** gave a less complicated mixture which contained debenzoylated oxime **15c** as the major component. Presumably, the persistent radical **8a** promotes cleavage of the benzoyl protecting group of both **12k** and **15b**, and the resulting radicals react in several ways to give a mixture of products. Using unprotected oxime **15c** alleviated this problem, and reaction of **12a** with nucleoside **15c** furnished the T*C^{Me} dimer **16b** in 75% yield. Next, we attempted the reaction of purine analog **12b** with **15a** which gave **16c** in 52% yield and attested to the stability of the acetamidine protecting group to these reaction conditions. Reaction of the 2′-*O*-methylguanosine-derived iodide **12c** with **15a** afforded a modest 40% yield of the G^{ibu}*T dimer **16d**, while reaction with **15c** gave 55% of the G^{ibu}*C dimer **16e**. The successful synthesis of **16c**−**e** was particularly satisfying, in that it demonstrated that this methodology is compatible with the use of 3′-iodopurines as radical precursors, expanding the scope of the reaction. When compound **12c** was reacted with **15d** under the same conditions, a lower yield (30%) of corresponding G^{ibu}*A dimer **16f** was obtained. Similarly, compound **16g** was obtained in 40% yield from **12b** and **15d**. A careful examination of the products revealed that the partial loss of protecting groups, such as isobutyryl and DMT was taking place, undoubtedly contributing to the reduction of isolated product.

In order to address this issue we subsequently utilized 5′-*O*-TBDPS protected 3′-iodo nucleosides **12e**−**g** as radical precursors. The coupling of iodide **12e** (1.2 equiv) and oxime **15a** (1.0 equiv) in the presence of **8** (3 equiv) was far less efficient than the corresponding reaction on the 2′-deoxy compounds (Table 1, entry 10), as a ca. 40% yield of the desired dimer was obtained, along with significant amounts of 3′-deoxygenated product and unreacted **15a**. However, when the reaction was performed with a 1:3:2 ratio of **12e**:**15a**:**8**, an 84% yield of dimer **16h** was obtained, along with unreacted oxime (95%, based on amount not converted to product). These conditions are identical to those which gave only a 50% yield of the DMT-protected dimer and demonstrate the superiority of 5′-silyl protection to 5′-DMT protection for this reaction. This excellent yield prompted us to use a similar procedure with purine nucleosides. Reaction **12f** and **12g** with **15d** and **15f** gave the desired MI-dimers **16i**−**k** in 30−40% yields. A partial loss of the dmf group was observed under the radical conditions, undoubtedly contributing to the lower yields, and made a tedious chromatographic purification necessary. The dimer **16i** was obtained in pure form; however, satisfactory elemental analyses for **16j** and **16k** could not be obtained. The FAB$^+$ MS of product obtained in the case of **16j** (homogeneous on TLC) shows an m/e 1176 (M + H$^+$, 100%), indicating the presence of the desired dimer, and also an m/e 1121 (50%), corresponding to material having lost a single dmf protecting group.

The structures of these 2′-*O*-methyl MI dimers **16** were confirmed by extensive NMR studies, and an interesting observation was revealed through 400 MHz ^{1}H NMR spectroscopy, which indicated the presence of a dimeric nucleosidic impurity. This impurity displayed resonances very similar to the major component (in many cases the product appears homogeneous by 200 MHz ^{1}H

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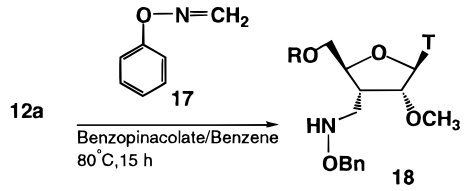
(44) Perbost, M.; Sanghvi, Y. S. *J. Chem. Soc., Perkin Trans. 1.* **1994**, 2051−2052.

Scheme 5



NMR), but was present in every dimer investigated, with the ratio varying for different dimers (5−25%). It should be pointed out that these compounds were all purified by column chromatography and were homogenous on TLC. We attribute this impurity to the presence of the *xylo* or *β*-diastereomer of the MI-dimer, having the inverted stereochemistry at the *C*-3′ of the upper nucleoside of the dimer. This result can be anticipated by the fact that during the homolytic fission of 3′-iodo nucleoside, a planar *C*-3′ radical is generated. The unpaired electron in a C-centered radical generally occupies an orbital which has mainly p-character,[45] therefore allowing attack from both faces. It is known in the literature that the presence of functional groups adjacent to the radical center exert a marked steric effect on the stereochemical outcome of the reaction, directing the incoming group to the most accessible lobe of the *π* radical.[46] Our results are consistent with this explanation, as for the 2′-deoxy series the α-face of the incipient *C*-3′ radical is unsubstituted, while the *β*-face is shielded by the nucleobase as well as the 5′-substituent. This results in the observed exclusive formation of the 3′-C-*ribo* dimer. For the *C*-3′ radical derived from a 2′-*O*-methyl nucleoside, however, the methoxy group adjacent to the radical center exerts a steric effect which counters that of nucleobase and the 5′-substituent, resulting in less selective attack of the incoming oxime and the observed mixtures of products.

The basis for the high stereoselectivity of radical addition reactions at the 3′-position of nucleosides has been attributed to the presence of bulky 5′-protecting groups as well as the nucleobase.[24a] We wished to investigate whether, if the steric constraint at the 5′-position was reduced, the resulting pinacolate-mediated coupling reaction would result in a higher isomeric ratio of the corresponding MI dimers. Toward this end, the iodide **12h** was reacted with **15a** under our standard conditions to furnish the T*T dimer **16l** in 68% yield. Surprisingly, the ratio of **16l** to the presumed isomeric impurity did not change significantly from the ca. 85:15 mixture of *ribo*- and *xylo*-diastereomers seen for **16h**. This suggests that the bulky group at the 5′-position has little or no effect on the stereochemical outcome during free radical-mediated C−C bond formation at the *C*-3′ position of nucleosides and indicates the nucleobase as the more important steric director.

We also sought to extend the one-carbon homologation reported by Hart[12] to the iodides **12**, which may be useful synthons for the preparation of biologically active nucleosides. Thus, the reaction of **12a** with *O*-benzylformaldoxine (**17**, 3 equiv) in degassed benzene at 80 °C in presence of **8** gave the 3′-(benzyloxyamino)methyl analog **18**, along with the corresponding 3′-epimer as a 5:1 mixture in 40% yield (Scheme 5). Potentially, a cleavage of the N−O bond or debenzylation of **18** would result in the synthesis of novel C-branched nucleosides. The ^{1}H

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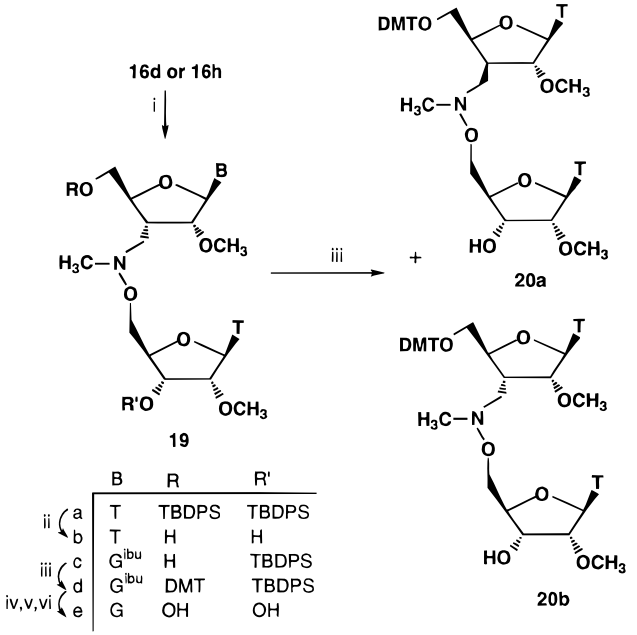
(46) Giese, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 753−764.

Scheme 6. Synthesis of 2′-*O*-Methyl MMI Dimers[a]



[a] Reagents and conditions: (i) HCHO/$NaBH_3CN$/AcOH, 45 min; (ii) $Et_3N{\cdot}3HF$/Et_3N/THF, rt, 24 h; (iii) DMTCl/DMAP/pyridine, rt, 24 h; (iv) NH_4OH/dioxane, rt, 24 h; (v) TBAF on silica gel/THF, rt, 24 h; (vi) 1,1,1,3,3,3-hexafluoro-2-propanol, rt, 3 h.

NMR spectrum of **18** is simplified relative to the dimers **16**, due to the presence of only a single carbohydrate moiety. This enabled us to conclusively prove the stereochemistry of both the major and minor components of **18** by employing 2D NMR techniques. TOCSY and NOESY spectra were used to assign each of the chemical shifts for the major and minor products in the one-dimensional ^{1}H spectrum. The spectrum of the major product was consistent only with a structure having the H3′ on the *β*-face of the furanose ring, shown by the strong NOE between H6 and H3′, thereby conclusively establishing the stereochemistry of the 3′-position as having the *ribo*-configuration. The spectrum of the minor product was consistent only with a structure having inverted stereochemistry at the 3′-position. No NOE was observed between H6 and H3′, whereas moderate NOE was observed from H3′ to both H1′ and the 2′-*O*-methyl group, as well as a strong NOE between H3′ and H4′.

Finally, the representative dimers **16h** and **16d** were converted to desired MMI-dimers (Scheme 6) by reacting with formaldehyde and sodium cyanoborohydride in acetic acid[8a] to give **19a** and **19c**, respectively. The resulting MMI dimer **19c** was treated with DMTCl to give **19d**, because the DMT protecting group was lost during methylation. Desilylation of **19a** gave the fully deprotected T*T dimer **19b**, at which point the ratio of epimers obtained *via* the pinacolate mediated coupling reaction to form **16h** could be readily established as 86:14 by integration of the H6 protons. These isomers were also inseparable, both by TLC and reverse phase HPLC. The ^{1}H NMR of the mixture **19b** was studied in D_2O at 310 K, and TOCSY and NOESY spectra were used to assign all resonances in the ^{1}H spectrum and study the stereochemistry of the isomers. The spectra were consistent with the proposed structure for the major isomer, two *β*-nucleosides connected 3′→5′ by an MMI linkage. This was demonstrated by moderate NOE's from the H1′ of the upper nucleoside unit to its own H2′, H4′, TH6, and the 2′-*O*-methyl. Also observed was NOE transfer

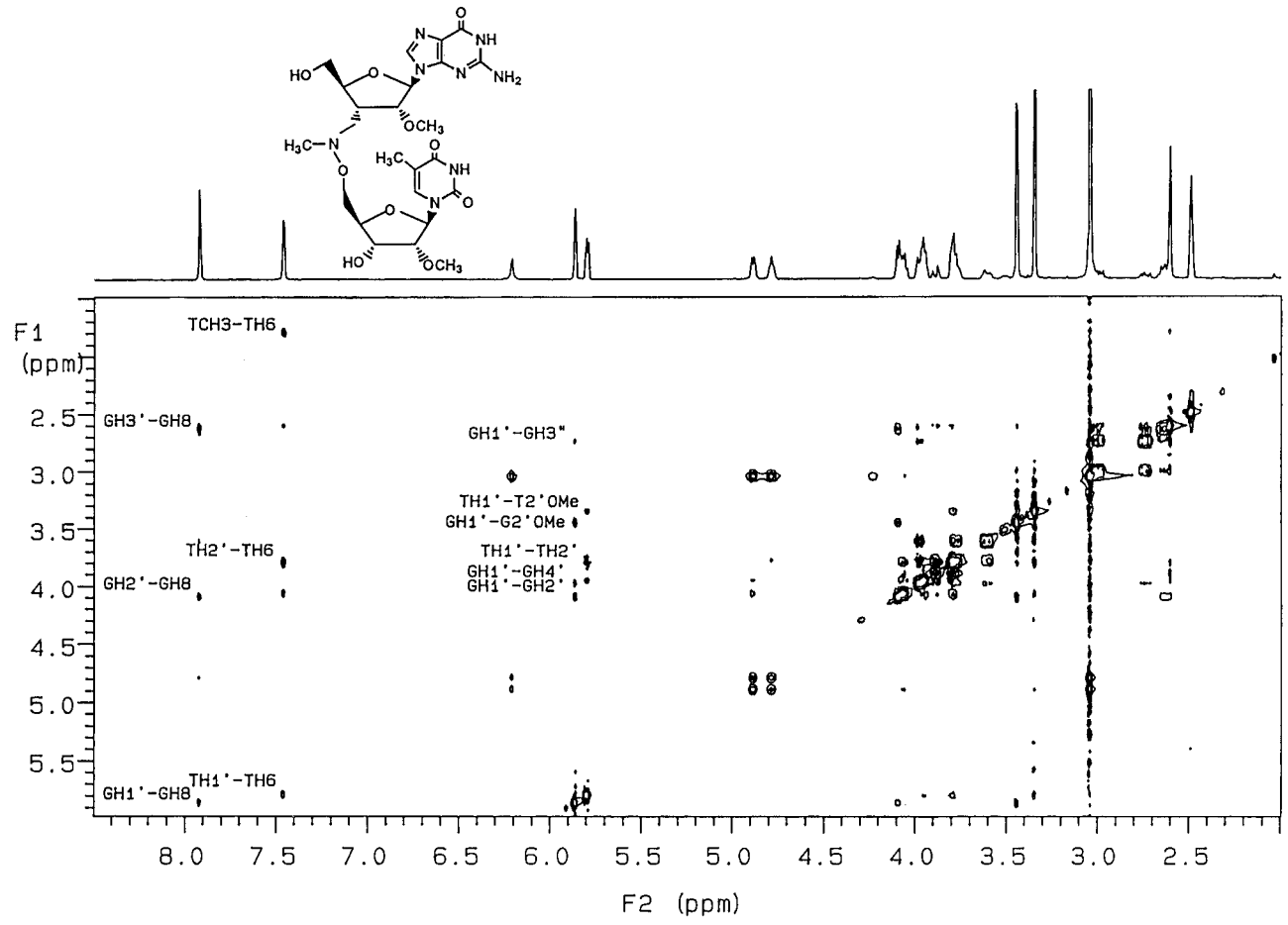


Figure 3. 2D NOESY spectrum of **19e**.

from the upper TH6 to H2′, H3′, and TCH_3. Moderate NOE is seen between the MMI *N*-methyl and the 2′-*O*-methyl of the upper nucleoside. In the lower nucleoside, NOE is observed from the H1′ to H2′, TH6, 2′-*O*-methyl and from TH6 to H2′, H3′, and TCH_3. As was the case for the mixture **18**, the key NOE contact is that observed between H6 and H3′ for the upper nucleoside, which unequivocally establishes the stereochemistry of the 3′-position of the major isomer as having the *ribo*-configuration.

The epimeric mixture of **19b** was treated with DMTCl in pyridine to give a 14:86 mixture of epimers **20a** and **20b**, respectively, which could be resolved on TLC, and were separable by careful column chromatography. TOCSY and NOESY spectra were used to assign the chemical shifts and to search for clues to define the chemical structure. For both compounds, it was necessary to run the spectra at 313 K in order to better increase chemical shift dispersion and allow for structure determination. For the upper nucleoside of the minor product **20a**, moderate-to-strong NOE′s were observed from H1′ to H2′, H3′, and H4′. The H6 proton showed a strong NOE to the H2′, and a weaker NOE to the H1′ and one of the 3′-methylene protons (H3″). The H1′ to H3′ NOE found for upper sugar of **20a** is consistent with that seen for the minor component in **18**, and unequivocally established the structure of the minor product in the pinacolate coupling reaction as the 3′-C-epimer, having the *xylo*-configuration at the 3′-position of the upper unit. The stereochemistry of the major product **20b**, although known from the NOESY of **19b**, could not be determined unambiguously from a 2D NMR experiment on **20b**. The upper furanose has strong NOE's from H6 to H2′ and TCH_3 and from H1′ to H2′, the 2′-*O*-methyl, H4′, and either the H3′ or H3″. The H3′ resonance of the upper sugar was superimposed upon one of the 3′-methylene protons (H3″), and therefore the observed NOE contact to this resonance from H6 cannot be used to determine the stereochemistry.

In order to perform detailed 2D NMR studies on the G*T MMI dimer without interference from protons of the protecting groups, the fully deprotected dimer **19e** was synthesized from **19d** through a series of deprotection reactions. It is noteworthy that the G*T dimer **16d** was formed in a 20:1 ratio over its corresponding 3′-epimer, which could be separated by chromatography, giving a single compound for study. The ^{1}H NMR of compound **19e** was studied in DMSO-d_6 solution at 293, 310, 333, and 353 K. TOCSY and NOESY (Figure 3) spectra at 310 and 353 K were used to assign all resonances in the ^{1}H spectrum. Only the 5′-5″ and 3′-methylene pairs remained without unequivocal definitions. The spectra were consistent with the proposed structure, as demonstrated by strong NOE's from GH8 to GH1′, GH2′, and GH3′, from GH2′ to GH3′ and GH1′, and from GH1′ to GH4′. The lower *ribo* showed positive NOE from TH6 to its H1′, H2′, and H3′, as well as between H1′ and H2′, H1′ and H4′, and from the 2′-*O*-methyl to H1′ and H2′. The experimental data was interpreted in terms of a two-state equilibrium between a North-type puckered ring and a South-type puckered ring using the PSEUROT methodology of Altona.[47] This methodology incorporates the vicinal coupling constants of ring protons as a means to estimate proton−proton torsion angles and then

Table 2. PSEUROT Calculations for the Sugar Conformations in 19e

	upper sugar (3′-methylene G-nucleoside)			lower sugar (T-nucleoside)	
	310 K	333 K	353 K	333 K	353 K
J (1′2′)	1.8	1.9	1.9	5.1	4.9
J (2′3′)	5.5	5.6	5.7	4.1	5.1
J (3′4′)	9.0	7.9	8.0	4.7	5.6
%N	90	77	78	46	52
%S	10	23	22	54	48
	$P_N = 36.6$; $P_S = 237.8$; $\phi_N = 34.0$; $\phi_S = 52.0$			$P_N = 20.4$; $P_S = 156.1$; $\phi_N = 38.0$; $\phi_S = 33.0$	

translates this information into the corresponding endocyclic torsion angle in terms of the pseudorotational parameters, phase angle (P), and maximum puckering amplitude (ϕ). The conformation of the two ribose rings of **19e** were analyzed on the basis of the vicinal J-couplings $^3J_{1'2'}$, $^3J_{2'3'}$, and $^3J_{3'4'}$. Standard parameters were adjusted to account for changes in the electronegativity and structure due to the 2′-methoxy moiety in both sugars and the MMI at the 3′-position.[48] Initial conditions for the PSEUROT calculation were suggested by previous work indicating that a ribonucleoside modified with a 3′-*ribo* C−C bond prefers to adopt a 3′-endo conformation.[21] Table 2 compiles the vicinal proton−proton coupling constants ($^3J_{HH}$) derived from those spectra for use in determining the conformation of the MMI-ribose ring, and the results of the PSEUROT calculations. It was not possible to determine all $^3J_{HH}$ values for each molecule at every temperature, and pseudorotation was only calculated where data was obtainable. These results support the hypothesis that the 2′-*O*-methyl-3′-*ribo*-CH_2-modified ribose ring (upper unit) strongly favors the north-type pucker, with 80−90% of the population adopting a C3′-endo form. The calculations for the lower unit support a pseudorotational equilibrium more typical of RNA, with 50% of the population in the northern conformation.

Conclusions

In summary, we have developed an efficient and high-yielding synthesis of nucleosidic radical precursors and acceptors and demonstrated the use of these synthons in a highly convergent radical coupling reaction to afford nucleosidic dimers. The 3′-iodo-2′-*O*-methyl nucleosides **12** were conveniently prepared from the corresponding unprotected nucleoside via activation of the 3′-hydroxyl as the triflate, followed by displacement with iodide. This reaction occurs with inversion of configuration at *C*-3′, giving the *xylo*-iodo nucleosides. These hitherto unknown iodo nucleosides are potentially versatile precursors in a variety of free radical as well as ionic transformations. An extremely efficient synthesis of the requisite 5′-*O*-methylene(imino) nucleosides **15** has also been developed, which relies on a regioselective Mitsunobu reaction of the 5′-hydroxyl group of the parent nucleosides. This reaction gives very high yields of the phthalimides **13** for the 5-methyluridine and adenosine derivatives, as well as for the dmf protected guanosine derivative. The use of the dmf protecting group on the guanine nucleobase results in exclusive reaction at the 5′-position, rendering the N1, N3, and O6 unreactive under Mitsunobu conditions, which may have profound implications for other transformations of guanine derivatives.[35] Use of the oximes **15** as traps for non-nucleosidic radicals has not been explored, but the addition of a variety of C-centered radicals can be envisaged, which would result in the formation of a variety of novel 5′-*O*-amino nucleoside derivatives.

Utilizing the pinacolate-mediated one-carbon homologation reported by Hart and Seely,[12] a highly convergent method for assembling complex nucleosidic dimers as mimics of natural nucleic acids has been developed. To our knowledge, this is the first example of the assembly of nucleosidic dimers *via* free radical methods, and although we have only investigated the assembly of dimers, it is feasible that higher order homologues could be constructed through this approach.[49] The results detailed herein describe the optimization of this reaction for the 2′-deoxy series, resulting in an improvement of yield of dimer **9a** from 25% to over 80%. This result compares very favorably with our previously reported route, which requires four transformations in 30% overall yield to obtain **9a**, whereas the pinacolate-mediated coupling reaction gives an 81% yield of identical material *in a single step*. The utility of this reaction for the preparation of mixed base dimers, in both the deoxy series and 2′-*O*-methyl series, has been demonstrated. Of special importance toward our goal of incorporating these dimers into antisense oligonucleotides **4** and **5**, is the ability to incorporate all four types of natural nucleosides into both the top and bottom portions of the dimers **9** and **16**. Our results suggest that the highest yields are obtained for homopyrimidine dimers, with fair and modest yields obtained for heterodimers and homopurine dimers, respectively.

In general, we found that reactions using 5′-*O*-TBDPS protected substrates were cleaner than those employing the corresponding 5′-*O*-DMT protected derivatives, making purification of dimers **9** or **16** easier. Furthermore, base protection is not required and is often detrimental to the success of the reaction. The use of unprotected nucleobases becomes a problem only in the case of guanine, as unprotected G derivatives are insoluble in benzene. This can be overcome by treating the crude product mixture with NH_4OH, so as to effect complete removal of the protecting group. This strategy is exemplified in the synthesis of **9e**. The reaction occurs with remarkable stereoselectivity, as no traces of any isomers resulting from β-face attack were evident for the 2′-deoxy series **9**. In the 2′-*O*-methyl series **16**, reasonable stereoselectivity is still maintained, even upon removal of the bulky 5′-protecting group, indicating that the β-linked nucleobase is the predominant steric factor governing the outcome of the reaction. Finally, as a representative example, the 2′-*O*-methyl dimer **16d** was converted to the corresponding MMI dimer and then completely deprotected to afford the unsubstituted G*T MMI dimer **19d**. The structure of this dimer was confirmed by ^{1}H NMR methods, and the pseudorotational parameters were calculated. The results indicate that both sugar units adopt predominantly an RNA-like sugar pucker, with the

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(48) (a) Altona, C.; Ippel, J. H.; Westra Hoekzema, A. J. A.; Erkelens, C.; Groesbeek, M.; Donders, L. A. *Magn. Reson. Chem.* **1989**, *27*, 564. (b) Altona, C.; Francke, R.; de Haan, R.; Ippel, J. H.; Daalmans, G. J.; Westra Hoekzema, A. J. A.; vanWijk, J. *Magn. Reson. Chem.* **1994**, *32*, 670.

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upper unit being essentially locked into the northern conformation. This suggests that *preorganized structures* of type **5** will adopt a conformation which is more favorable for hybridization with the target mRNA, potentially increasing their affinity, specificity, and thereby their usefulness in the design of superior antisense oligonucleotides.

Experimental Section

General. Unless otherwise noted, materials were obtained from commercial suppliers and were used as provided. 2′-*O*-Methyl-5-methyluridine, 5′-*O*-(4,4′-dimethoxytriphenylmethyl)-2′-*O*-methyl-5-methyluridine, 5′-*O*-(4,4′-dimethoxytriphenylmethyl)-*N*-2-isobutyryl-2′-*O*-methylguanosine, and *N*-6-benzoyl-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-2′-*O*-methyladenosine were purchased from RI Chemicals, CA (714-288-1548). 2′-*O*-Methylguanosine and 2′-*O*-methyladenosine were purchased from Summit Pharmaceuticals Corp., N.J. (201-585-9687). Other general experimental procedures were carried out as described previously.[26]

NMR Spectroscopy. ^{1}H NMR spectra for all compounds were recorded either at 399.94 MHz on a Varian Unity 400 NMR spectrometer or at 199.975 MHz on a Varian Gemini 200 NMR spectrometer. All multidimensional and variable temperature spectra were collected on the Unity 400 NMR. The compound **19d** was studied at a concentration of 1.6 mM in 600 μL of DMSO-d_6. One-dimensional ^{1}H NMR spectra were recorded at the sample temperatures 293, 310, 333, and 353 K. All one-dimensional measurements were performed under the same conditions and processed identically: 5200 Hz sweep width, 32K time domain, zero-filling to 64K. Where possible, the data were studied unapodized, but in some instances the standard Varian VnmrS resolution enhancement method was invoked, using a line broadening of −1.01 and a gaussian filter of 0.945. All subspectra were observed to be first order. Proton resonances were assigned from two-dimensional spectra run at 310 and 353 K. TOCSY and NOESY spectra were run at 310 K with a 20 ms spin-lock and a 650 ms mixing time, respectively, with 1K by 512 complex points being collected in a phase-sensitive manner. Similar experiments were carried out at 353 K, with 2K by 512 complex points collected in a phase-sensitive manner. In each case, the data was apodized with a squared cosine and zero-filled to 2K × 1K complex points before Fourier transformation. The ^{13}C spectra were recorded on the same instrument, at a frequency of 100.57 MHz, using a sweep width of 30441 Hz, an acquisition time of 0.50 s, and processed by apodizing with 2 Hz line-broadening and zero-filling to 32K. The ^{13}C spectrum was assigned with the aid of a ^{1}H{^{13}C} HMQC at 293 K. For this experiment 1K complex points were collected in the directly-detected dimension over a sweep width of 3200 Hz. A total of 240 increments were collected in a phase-sensitive manner in the indirectly-detected dimension, over a sweep-width of 22 kHz. The data was apodized with a squared cosine in the ^{1}H dimension and a gaussian in t_1.

One-dimensional ^{1}H spectra for **12h**, **12i**, **12j**, **18a**, **20a**, and **20b** were all recorded at 293 K using identical parameters for acquisition and processing: 14336 complex points were collected over a sweep width of 7 kHz. Data was zero-filled to 64K points and apodized with 0.30 Hz line-broadening before Fourier transform. Two-dimensional spectra were acquired with sweep widths adjusted to optimize resolution across the spectral range of each sample, ranging from 4 kHz to 5200 Hz in each dimension. Data sets of 1K by 256 points were acquired for **12h** and **12j**, 1K by 512 points for **12i** and **20b.** Data sets of 2K by 512 data points were collected for **18** and **20b** to allow adequate resolution**.** Data was collected at 313 K for **20a** and **20b** to enhance chemical shift dispersion. All data was phase-sensitive, processed under conditions otherwise identical to the methods used in the TOCSY and NOESY work outlined above. ^{1}H NMR data for **19b** was collected at 310 K in deuterium oxide, using the same series of experiments described above.

General Procedure A. Synthesis of 3′-Iodo-2′-*O*-methyl Nucleosides (11 → 12). To a solution of pyridine (4.3 equiv) in dry CH_2Cl_2 (5 mL/mmol) at −10 °C under an inert atmosphere was added a solution of trifluoromethanesulfonic anhydride (2 equiv) in CH_2Cl_2 (5 mL/mmol) dropwise over 0.5 h. After stirring an additional 0.25 h, a solution of appropriately protected 3′-hydroxyl nucleoside (1 equiv, azeotroped with dry pyridine) in dry CH_2Cl_2 (5 mL/mmol) was added dropwise over 0.5 h, and the solution was stirred at −10 °C for an additional 2 h. The reaction mixture was diluted with an equal volume of ice cold 10% aqueous $NaHCO_3$ with vigorous stirring, and the mixture was allowed to warm to rt. The organic phase was removed, dried ($MgSO_4$), diluted with toluene (5 mL/mmol), concentrated, and azeotroped with dry toluene (3 × 5 mL/mmol). To the resulting foam were added Bu_4NI (2 equiv) and dry toluene (30 mL/mmol), and the resulting mixture was heated for 0.5 h at 80 °C in an oil bath with vigorous stirring, at which point all solid had dissolved and a dark red oil separated. The resulting mixture was dissolved by dilution with EtOAc and washed with water (15 mL/mmol), 1:1 5% aqueous $NaHSO_3$ and 5% aqueous Na_2SO_3 (2 × 15 mL/mmol), dried ($MgSO_4$), concentrated, and chromatographed to provide the iodide.

3′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-3′-iodo-2′-*O*-methyl-5-methyluridine (12a). From 5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-methyl-5-methyluridine (5.75 g, 10 mmol) according to general procedure A was obtained 4.41 g (64%) of **12a** after chromatography (30% to 50% EtOAc/hexane): R_f 0.38 (50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 9.91 (s, 1H), 7.55−7.20 (m, 10H), 6.85 (d, 4H), 5.85 (s, 1H, H-1′), 4.37 (s, 1H), 4.31 (d, 1H), 3.93 (m, 1H), 3.79 (s, 6H), 3.60−3.50 (m, 1H), 3.56 (s, 3H), 3.20 (m, 1H), 1.83 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 164.16, 158.53, 150.35, 144.23, 136.14, 135.44, 135.25, 130.06, 128.12, 127.77, 126.88, 113.06, 110.06, 92.48, 90.17, 86.55, 80.00, 68.56, 58.32, 55.09, 26.84, 12.32. Anal. Calcd for $C_{32}H_{33}N_2O_7I\cdot0.4CH_2Cl_2$: C, 54.16; H, 4.74; N, 3.90. Found: C, 54.27; H, 4.58; N, 3.73.

3′-Deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-3′-iodo-2-*N*-isobutyryl-2′-*O*-methylguanosine (12c). From 5 g (8.26 mmol) of **11c** according to general procedure A was obtained 3.8 g (59%) of **12b** after chromatography (80% EtOAc/hexane): ^{1}H NMR (DMSO-d_6) δ 12.14 (s, 1H), 11.67 (s, 1H), 7.96 (s, 1H), 7.42−7.20 (m, 9H), 7.17−6.86 (m, 4H), 5.85 (d, J = 2.1 Hz, 1H), 4.74 (bs, 2H), 3.87−3.86 (m, 1H), 3.73 (s, 6H), 3.32−3.08 (m, 5H), 2.85−2.70 (m, 1H), 1.12−1.03 (m, 6H); MS (FAB$^+$) m/e 780 (M + H). Anal. Calcd for $C_{36}H_{38}N_5O_7I\cdot0.25C_6H_{14}$: C, 56.25; H, 5.16; N, 8.75. Found: C, 56.48; H, 5.24; N, 8.68.

6-*N*-Benzoyl-3′-deoxy-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-3′-iodo-2′-*O*-methyladenosine (12k). From *N*-6-benzoyl-5′-*O*-(4,4′-dimethoxytrityl)-2′-*O*-methyladenosine (0.69 g, 1 mmol) according to general procedure A was obtained 0.47 g (58%) of **12k** after chromatography (50% to 70% EtOAc/hexane): R_f 0.34 (50% EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 9.12 (s, 1H), 8.81 (s, 1H), 8.37 (s, 1H), 8.03 (d, 2H), 7.65−7.15 (m, 9H), 6.85 (d, 4H), 6.18 (s, 1H, H-1′), 4.68 (s, 1H), 4.39 (d, 1H), 3.94 (dd, 1H), 3.80 (s, 6H), 3.66 (dd, 1H), 3.63 (s, 3H), 3.21 (dd, 1H); ^{13}C NMR ($CDCl_3$) δ 164.52, 158.61, 158.57, 152.71, 151.09, 149.37, 144.36, 141.46, 135.64, 135.35, 133.61, 132.72, 130.10, 130.03, 128.81, 128.10, 127.86, 127.79, 126.96, 123.38, 113.16, 92.76, 89.73, 86.61, 80.48, 68.58, 58.38, 55.20, 26.60. Anal. Calcd for $C_{39}H_{36}N_6O_6I\cdot0.4CH_2Cl_2\cdot0.5EtOAc$: C, 56.79; H, 4.70; N, 8.00. Found: C, 56.60; H, 4.64; N, 8.01.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-3′-iodo-5-methyl-2′-*O*-methyluridine (12e). A solution of **11e** (7.0 g, 13.7 mmol) and dry pyridine (1.9 g, 24.6 mmol) in anhydrous CH_2Cl_2 (80 mL) was cooled under vigorous stirring to −10 °C in an inert atmosphere for about 20 min. A solution of triflic anhydride (5.1 g, 17.7 mmol) in CH_2Cl_2 (30 mL) was slowly added via a syringe (15 min.). The reaction was complete in 35 min after the addition. To this cold solution was added MeOH (1 mL) and then saturated $NaHCO_3$, and the organic layer was separated and washed with brine (2 × 15 mL) followed by drying over Na_2SO_4. Solvent was removed under reduced pressure and the residue azeotroped with toluene (2 × 20 mL). The residual gum was dissolved in *p*-xylene (100 mL), to this was added Bu_4NI (5.6 g,15.1 mmol), and the reaction mixture was heated at 140 °C in a preheated bath.

The reaction was complete in 30 min. It was cooled to rt and then diluted with EtOAc (250 mL) and washed with 5% aqueous solution of sodium sulfite and sodium bisulfite (1:1). The organic layer was dried over Na_2SO_4 and concentrated followed by flash chromatography on silica gel using 60% EtOAc/hexane. Removal of the solvent furnished 6.8 g (80%) of **12e** as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 11.50 (s, 1H), 7.23−7.43 (m, 11H), 5.78 (d, J = 4.0 Hz, 1H), 4.6 (bs, 1H), 4.40−4.43 (m, 1H), 3.89 (bs, 2H), 3.42 (s, 3H), 1.59 (s, 3H), 1.04 (s, 9H); MS (FAB$^+$) m/e 621 (M + H). Anal. Calcd for $C_{27}H_{33}N_2O_5SiI$: C,52.26; H, 5.36; N, 4.51. Found: C, 52.55; H, 5.41; N, 4.48.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-6-*N*-[(dimethylamino)methylene]-3′-iodo-2′-*O*-methyladenosine (12f). From **11h** (5.3g, 9.2 mmol), pyridine (3.65 g, 46.1 mmol), triflic anhydride (3.0 g, 11 mmol), and Bu_4NI (5.2 g, 14.0 mmol) according to the method used for **12e** was obtained 3.8 g (60%) of **12f** after chromatography on silica gel using 80:15:3−5% MeOH in EtOAc:hexane: ^{1}H NMR (DMSO-d_6) δ 8.93 (s, 1H), 8.45 (s, 1H), 8.24 (s, 1H), 7.73−7.39 (m, 10H), 6.07 (d, J = 2.8 Hz, 1H), 4.89−4.87 (m, 1H), 4.76−4.74 (m, 1H), 3.98−3.93 (m, 3H), 3.46 (s, 3H), 3.21 (s, 3H), 3.14 (s, 3H), 1.03 (s, 9H); MS (FAB$^+$) m/e 685 (M + H). Anal. Calcd for $C_{30}H_{37}N_6O_3SiI\cdot 0.25C_6H_{12}$: C, 53.61; H, 5.71; N, 11.91. Found: C, 53.20; H, 5.58; N, 12.24.

5′-*O*-(*tert*-Butyldiphenylsilyl)-3′-deoxy-2-*N*-[(dimethylamino)methylene]-3′-iodo-2′-*O*-methylguanosine (12g). From **11j** (5.0 g, 8.46 mmol), pyridine (3.36 g, 46 mmol), triflic anhydride (3 g, 11 mmol), and Bu_4NI (5.2 g, 14.0 mmol) according to the method used for **12e** was obtained 2.5 g (43%) of **12g** after chromatography on silica gel using 80:15:3−5% MeOH in EtOAc:hexane: ^{1}H NMR (DMSO-d_6) δ 11.43 (s, 1H), 8.58 (s, 1H), 7.87 (s, 1H), 7.70−7.64 (m, 10H), 5.90 (d, J = 2 Hz, 1H), 4.88 (bs, 1H), 4.70 (bs, 1H), 3.46 (s, 3H), 3.12 (s, 3H), 3.04 (s, 3H), 1.02 (s, 9H; MS (FAB$^+$) m/e 701 (M + H). Anal. Calcd for $C_{30}H_{37}N_6O_4SiI$: C, 51.43; H, 5.32; N, 11.99. Found: C, 51.56; H, 5.37; N, 11.98.

General Procedure B. Mitsunobu Reaction of Nucleosides (10 → 13). A mixture of the appropriate nucleoside (1 equiv), triphenylphosphine (1.15 equiv), and *N*-hydroxyphthalimide (1.15 equiv) was dried under vacuum over P_2O_5 for 18 h prior to use. To this mixture under an inert atmosphere is added dry THF (for T) or DMF (for A and G^{dmf}) (10 mL/mmol) and then diethyl azodicarboxylate (1.15 equiv) dropwise with stirring to the solution at rt. The rate of addition is maintained such that the resulting deep red coloration is just discharged before addition of the next drop. After the addition is complete (5−45 min, depending on starting nucleoside and scale), the reaction is stirred until shown to be complete by TLC. The solvent was evaporated *in vacuo* to approximately 1/3 the starting volume, and if a solid was obtained (T, G^{dmf}) it was collected, otherwise (A) the resulting solution was partitioned between water and ether and the resulting solid collected, washed well with ether, and dried to yield the desired product. This material was pure in some cases, but usually contained traces of triphenylphosphine oxide and/or diethyl hydrazinedicarboxylate, which could be removed by column chromatography. However, these impurities did not interfere with the subsequent reactions (general procedure C), and the crude material could therefore be carried directly to the next step.

2′-*O*-Methyl-5′-*O*-phthalimido-5-methyluridine (13a). 2′-*O*-Methyl-5-methyluridine (4.08 g, 15 mmol) was reacted according to general procedure B. After the reaction was complete (by TLC), the solids were collected, washed well with ether, and dried to yield 3.06 g (49%) of pure product. The combined filtrates were concentrated then purified by column chromatography (0 to 4% MeOH/CH_2Cl_2) to provide an additional 2.50 g (40%), giving a combined yield of 5.56 (89%) of **13a**: R_f 0.25 (5% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 11.36 (s, 1H), 7.87 (br s, 4H), 7.58 (s, 1H), 5.89 (d, J = 5.7 Hz, 1H), 5.43 (d, J = 5.7 Hz, 1H), 4.5−3.8 (m, 6H), 3.34 (s, 3H), 1.79 (s, 3H). Anal. Calcd for $C_{19}H_{19}N_3O_8\cdot 0.5H_2O$: C, 53.52; H, 4.73; N, 9.85. Found: C, 53.51; H, 4.49; N, 9.84.

2′-*O*-Methyl-5′-*O*-phthalimidoadenosine (13b). From 2′-*O*-methyladenosine (50.0 g, 178 mmol) according to general procedure B was obtained 74.3 g (98%) of product after partitioning the concentrated reaction mixture between ice water (3 L) and ether (3 L) and collection and drying of the resulting solid. The product contains traces (0.125 equiv by ^{1}H NMR and combustion analysis) of $Ph_3P{=}O$, which does not interfere in the silylation reaction: R_f 0.38 (10% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.36 (s, 1H), 8.12 (s, 1H), 7.81 (s, 4H), 6.02 (d, J = 5.0 Hz, 1H), 5.50 (d, J = 5.0 Hz, 1H), 4.6−4.3 (m, 4H), 4.3−4.2 (m, 1H), 3.33 (s, 3H); HRMS (FAB$^+$, CsI/NBA) calcd for $C_{19}H_{18}N_6O_6 + Cs^+$ 559.0342, found 559.0355. Anal. Calcd for $C_{19}H_{18}N_6O_6\cdot 0.125Ph_3P{=}O$ (evident in ^{1}H NMR): C, 55.34; H, 4.34; N, 18.22. Found: C, 55.07; H, 4.45; N, 17.98.

2-*N*-[(Dimethylamino)methylene]-2′-*O*-methyl-5′-phthalimidoguanosine (13c). 2-*N*-[(Dimethylamino)methylene]-2′-*O*-methylguanosine (**10f**, 6.39 g, 18.2 mmol) was exhaustively dried (0.01 mmHg, 45 °C, several days over P_2O_5) and then reacted according to general procedure B. After the reaction was complete (by TLC), it was reduced to 1/3 its original volume, and the solid was collected and dried to yield 8.85 g (93%) of hydrated **13c:** R_f 0.23 (10% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 11.39 (s, 1H), 8.53 (s, 1H), 8.05 (s, 1H), 7.84 (br s, 4H), 5.92 (d, J = 5.8 Hz, 1H), 5.52 (d, J = 5.3 Hz, 1H), 4.5−4.1 (m, 5H), 3.14 (s, 3 H), 3.01 (s, 3H), 2.88 (s, 3 H); HRMS (FAB$^+$, CsI/NBA) calcd for $C_{22}H_{23}N_7O_7 + Cs^+$ 630.0713, found 630.0725. Anal. Calcd for $C_{22}H_{23}N_7O_7\cdot 1.5H_2O$: C, 50.38; H, 5.00; N, 18.69. Found: C, 50.57; H, 5.12; N, 18.81.

General Procedure C. Silylation of Nucleosides (13 → 14). The requisite nucleoside (1 equiv, dried over P_2O_5 *in vacuo*) and imidazole (4.5 equiv) were dissolved in dry DMF (5 mL/mmol) under an inert atmosphere, and TBDPSCl (1.5 equiv) was added. The reaction was stirred at room temperature until complete by TLC (16−48 h), then poured into EtOAc (15 mL/mmol) and water (15 mL/mmol). The organic layer was washed with water (3 × 10 mL/mmol), dried ($MgSO_4$), and concentrated. On large scale, it proved more convenient to concentrate the crude reaction mixture to a thin syrup, partition the product between ether and water, and then collect the resulting solid. This material could be purified by column chromatography; however, the only impurities present were silyl byproducts, which did not interfere in the subsequent conversion to the oximes *via* general procedure D.

3′-(*tert*-Butyldiphenylsilyl)-2-*N*-[(dimethylamino)methylene]-2′-*O*-methylguanosine (14c). From **13c** (8.85 g, 16.9 mmol) according to general procedure C was obtained 10.6 g (84%) of **14c** after filtration (0 to 10% MeOH/CH_2Cl_2) of the crude material through a plug of silica: R_f 0.36 (10% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H), 8.43 (s, 1H), 8.04 (s, 1H), 7.84 (br s, 4H), 7.5−7.7 (m, 4H), 7.3−7.4 (m, 6H), 6.02 (d, J = 6.1 Hz, 1H), 4.5−4.7 (m, 1H), 4.0−4.5 (m, 4H) 3.05 (s, 3 H), 3.03 (s, 3H), 2.88 (s, 3 H), 1.06 (s, 9H); HRMS (FAB$^+$, CsI/NBA) calcd for $C_{38}H_{41}N_7O_7Si + Cs^+$ 868.1891, found 868.1899. Anal. Calcd for $C_{38}H_{41}N_7O_7Si\cdot 0.5H_2O$: C, 61.27; H, 5.68; N, 13.16. Found: C, 61.27; H, 5.82; N, 13.38.

General Procedure D. Synthesis of Nucleoside 5′-Formaldoximes (14 → 15). The crude material obtained from the silylation of the corresponding 5′-*O*-phthalimido nucleoside was dissolved in dry CH_2Cl_2 (10 mL/mmol), and methylhydrazine (1.2 equiv) was added dropwise at −10 to 0 °C. After 1 h, the mixture was filtered, the filtrates were washed with CH_2Cl_2, the combined organics were washed with water and brine and dried (Na_2SO_4), and the solution was concentrated to yield the 5′-*O*-amino nucleoside, which was used immediately without further purification. (The 5′-*O*-amino nucleosides can be purified by column chromatography; however, we have observed that they tend to decompose upon standing). This residue was dissolved in 1:1 EtOAc/MeOH (15 mL/mmol), formaldehyde (20% w/w aqueous, 1.1 equiv) was added, and the mixture was stirred 1 h at room temperature. The solution was concentrated and then chromatographed or crystallized to provide the 3′-silyl-5′-formaldoxime.

3′-*O*-(*tert*-Butyldiphenylsilyl)-2-*N*-[(dimethylamino)methylene]-2′-*O*-methyl-5′-*O*-(methyleneimino)guanosine (15e). From **14c** (10.6 g, 14.4 mmol) according to general procedure D was obtained 9.21 g of **15e** in essentially quantitative yield after filtration (0 to 10% MeOH/CH_2Cl_2) of

the crude material through a plug of silica: R_f 0.43 (10% MeOH/CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 11.19 (s, 1H), 8.47 (s, 1H), 7.94 (s, 1H), 7.5−7.7 (m, 4H), 7.4−7.5 (m, 6H), 6.96 (d, J = 7.27 Hz, 1H), 6.55 (d, J = 7.27 Hz, 1H), 6.02 (d, J = 6.13 Hz, 1H), 4.4−4.5 (m, 1H), 4.0−4.3 (m, 4H) 3.06 (s, 3 H), 3.01 (br s, 6H), 1.06 (s, 9H) ppm. Anal. Calcd for $C_{31}H_{39}N_7O_5Si\cdot 0.5H_2O$: C, 59.40; H, 6.43; N, 15.64. Found: C, 59.59; H, 6.33; N, 15.61.

General Procedure E. Radical Coupling Reaction. The appropriate 3′-deoxy-3′-iodo nucleoside (1 equiv) and 5′-formaldoxime (3 equiv) were azeotroped with dry benzene (10 mL/mmol), and bis(trimethylstannyl) benzopinacolate (2 equiv) and dry benzene (5 mL/mmol) were added. The mixture was degassed (argon, 0.5 h) and heated to 80 °C in an oil bath for 16−24 h, at which point all iodide had been consumed (by TLC). The solution was allowed to cool and stirred vigorously with EtOAc (50 mL/mmol) and 10% aqueous KF (20 mL/mmol) for 2 h. The organic layer was washed with 5% aqueous $NaHCO_3$ (20 mL/mmol), dried ($MgSO_4$), concentrated, dissolved in the minimum amount of CH_2Cl_2, and applied to a column of silica. Elution with 30% EtOAc/hexane (5 column volumes) provided tin byproducts and benzophenone. The unreacted oxime (75−99% of unreacted material recovered) and the hydroxylamino-linked dimer were then obtained by continued elution with the appropriate solvent system.

3′-De(oxyphosphinico)-3′-(methyleneimino)-5′-*O*-(triphenylmethyl)thymidylyl-(3′→5′)-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-deoxyadenosine (9d). From 297 mg (0.5 mmol) of **6b** and 775 mg (1.5 mmol) of **7b** according to general procedure F was obtained 515 mg (83% of unreacted material) of the starting oxime, and 291 mg (59%) of **9d** after elution of the column with a gradient of 0% to 5% MeOH in 4:1 EtOAc/hexane: R_f 0.41 (5% MeOH in 4:1 EtOAc/hexane); ^{1}H NMR ($CDCl_3$) δ 12.50 (s, 1H), 8.34 (s, 1H), 8.23 (s, 1H), 7.70−7.10 (m, 26H), 6.55 (t, J = 6.8 Hz, 1H), 6.45 (bs, 2H), 6.08 (t, J = 5.6 Hz, 1H), 5.98 (s, 1H), 4.50 (m, 1H), 4.07 (m, 1H), 3.94 (m, 1H), 3.47 (m, 2H), 3.28 (dd, 1H), 3.20 (dd, 1H), 2.84 (m, 2H), 2.45 (m, 3H), 2.19 (t, 2H), 1.52 (s, 1H), 1.09 (s, 9H). Anal. Calcd for $C_{56}H_{60}N_8O_7Si\cdot 0.5H_2O$: C, 67.65; H, 6.18; N, 11.27. Found: C, 67.77; H, 6.06; N, 11.35.

3′-De(oxyphosphinico)-3′-(methyleneimino)-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-methyl-5-methyluridine (16h). Compound **12e** (1.22 g, 1.64 mmol), **15a** (2.5 g, 4.15 mmol), and **8** (2.26 g, 3.27 mmol) in benzene (16 mL) were reacted according to general procedure E. Continued elution of the column with 50% EtOAc/hexane (10 column volumes) gave the unreacted oxime **15a** (1.80 g, 95% of unreacted material), and elution with 10% MeOH/CH_2Cl_2 afforded 1.42 g (84%) of the hydroxylamino linked dimer **16h**: R_f 0.15 (5% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 8.83 (s, 1H), 8.67 (s, 1H), 7.80−7.15 (m, 22H), 5.91 (s, 1H), 5.84 (d, J = 2.3 Hz, 1H), 5.55 (t, 1H), 4.30−3.00 (m, 10H), 3.55 (s, 3H), 3.32 (s, 3H), 2.87 (m, 1H), 2.46 (m, 1H), 1.76 (s, 3H), 1.48 (s, 3H), 1.10 (s, 9H), 1.09 (s, 9H). Anal. Calcd for $C_{55}H_{69}N_5O_{11}Si_2$: C, 63.99; H, 6.74; N, 6.78. Found: C, 63.65; H, 6.64; N, 6.58.

General Procedure F. Reductive Methylation of MI Dimers. The appropriate *base-unprotected* MI dimer (1 equiv) was dissolved in AcOH (10 mL/mmol) and cooled in a cool water bath (not to freezing), and $NaBH_3CN$ (2 equiv) was added in one portion, with vigorous stirring. To this suspension was added formaldehyde (20% aqueous, 15 equiv) in a slow stream, the mixture was stirred for 5 min, and additional portions of $NaBH_3CN$ (2 × 2 equiv) were added portionwise over 10 min. The cool bath was removed, and the resulting solution was allowed to warm to room temperature with stirring over 1 h. The reaction mixture was poured into ice cold water (50 mL/mmol), the resulting material was dissolved with EtOAc (15 mL/mmol), and the organic phase was washed with water (2 × 50 mL/mmol), 10% aqueous $NaHCO_3$ (carefully, gas evolution) until the washings remained pH = 8 (typically 1 × 15 mL/mmol), water (50 mL/mmol), and brine and then dried ($MgSO_4$). The solvent was removed, and the syrup was azeotroped (2×) with dry MeCN to provide a foam and then dried in vacuo.

3′-De(oxyphosphinico)-3′-[methylene(methylimino)]-5′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-methyl-5-methyluridine (19a). From 4.1 g (4.0 mmol) of **16h** according to general procedure F was obtained 2.93 g (70%) of the desired MMI dimer, which required no chromatographic purification: R_f 0.65 (10% MeOH/CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 8.94 (s, 1H), 8.84 (s, 1H), 7.80−7.15 (m, 22H), 5.89 (s, 1H), 5.75 (s, 1H), 4.30−2.85 (m, 10H), 3.55 (s, 3H), 3.29 (s, 3H), 2.55−2.35 (m, 2H), 2.49 (s, 3H), 1.79 (s, 3H), 1.39 (s, 3H), 1.09 (s, 18H). Anal. Calcd for $C_{56}H_{71}N_5O_{11}Si_2\cdot H_2O$: C, 63.19; H, 6.91; N, 6.58. Found: C, 63.32; H, 6.83; N, 6.64.

3′-De(oxyphosphinico)-3′-[methylene(methylimino)]-2-*N*-isobutyryl-2′-*O*-methylguanosylyl-(3′→5′)-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-methyl-5-methyluridine (19c). From 0.50 g (0.42 mmol) of **16d** according to general procedure F was obtained 0.35 g (92%) of the detritylated MMI dimer after chromatographic purification (70:15:15 EtOAc/hexane/MeOH): ^{1}H NMR ($CDCl_3$) δ 12.20 (bs, 1H), 10.05 (bs, 1H), 9.80 (bs, 1H), 8.00 (s, 1H), 7.71−7.20 (m, 11H), 5.82 (m, 2H), 4.64 (bs, 1H), 4.13−2.60 (m, 21H), 2.49 (s, 3H), 1.82 (s, 3H), 1.22 (s, 6H), 1.08 (s, 9H); MS (FAB$^+$) m/e 903 (M + H). Anal. Calcd for $C_{44}H_{57}N_8O_{11}Si\cdot 0.25H_2O$: C, 58.29; H, 6.39; N, 12.36. Found: C, 57.95; H, 6.57; N, 12.14.

3′-De(oxyphosphinico)-3′-[methylene(methylimino)]-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-2′-*O*-methyl-5-methyluridine (19b). To a solution of **19a** (2.93 g, 2.68 mmol) in dry THF (25 mL) were added $Et_3N\cdot 3HF$ (4.4 mL, 26.8 mmol) and Et_3N (1.9 mL, 13.4 mmol). The resulting solution was stirred at room temperature overnight, concentrated (0.1 mmHg) to a viscous syrup, chromatographed (0 to 10% MeOH/EtOAc), and then azeotroped with ethanol to provide 1.32 g (86%) of **19b** as a foam: R_f 0.40 (10% MeOH/EtOAc); ^{1}H NMR (DMSO-d_6) δ 11.35 (s, 1H), 11.28 (s, 1H), 8.05 (s, 1H), 7.93 (s, 0.16H, minor isomer present in 14:86 ratio), 7.54 (s, 1H), 5.82 (d, 1H), 5.75 (s, 1H), 5.25 (t, 1H), 5.19 (d, 1H), 4.20−3.50 (m, 9H),3.40 (s, 3H), 3.28 (s, 3H), 2.91 (m, 1H), 2.67 (m, 1H), 2.57 (s, 3H), 2.43 (m, 1H), 1.78 (s, 3H), 1.73 (s, 1H). Anal. Calcd for $C_{24}H_{35}N_5O_{11}\cdot 0.5EtOH$: C, 50.67; H, 6.46; N, 11.82. Found: C, 50.36; H, 6.25; N, 11.60.

3′-De(oxyphosphinico)-3′-[methylene(methylimino)]-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-2′-*O*-methyl-5-methyluridylyl-(3′→5′)-2′-*O*-methyl-5-methyluridine (20b) and Its *C*-3′ Epimer (20a). The dimer **19b** (1.70 g, 2.98 mmol) was azeotroped with dry pyridine (3×), dissolved in the minimum amount of pyridine, and cooled to 0 °C, and 4,4′-dimethoxytrityl chloride (1.53 g, 4.5 mmol) was added in 3 portions over 4 h. The reaction mixture was allowed to come to rt with stirring overnight and then partitioned between CH_2Cl_2 (50 mL) and 5% $NaHCO_3$ (100 mL). The aqueous layer was washed with CH_2Cl_2, and the combined organics were washed with water and then dried ($MgSO_4$), concentrated, and azeotroped twice with toluene. The resulting residue was chromatographed (0 to 5% MeOH/CH_2Cl_2) to provide 2.32 g (89%) of a ca. 14:86 mixture of dimers (by ^{1}H NMR): R_f 0.53 (10% MeOH/CH_2Cl_2). 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, 61.27; H, 6.13; N, 8.03. A portion of this mixture (0.5 g) was carefully chromatographed (5 × 15 cm, 2% MeOH in 4:1 EtOAc−hexane) to provide the C-3′-epimer **20a**: R_f 0.18 (2% MeOH in 4:1 EtOAc−hexane); ^{1}H NMR ($CDCl_3$) δ 8.64 (s, 1H), 8.53 (s, 1H), 7.75 (s, 1H), 7.48 (s, 1H), 7.40−7.20 (m, 9H), 6.84 (m, 4H), 6.05 (d, J = 6.0 Hz, 1H), 5.86 (s, 1H), 4.43 (t, 1H), 4.07 (m, 2H), 3.92 (m, 1H), 3.88 (s, 6H), 3.87−3.69 (m, 3H), 3.60 (s, 3H), 3.39 (s, 1H), 2.82 (m, 1H), 2.74 (m, 1H), 2.66 (m, 1H), 2.16 (s, 3H), 1.91 (s, 3H), 1.12 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 163.54, 158.92, 150.40, 149.87, 143.29, 136.03, 135.35, 134.55, 130.49, 130.16, 129.10, 128.77, 127.94, 127.83, 127.74, 127.51, 113.20, 111.64, 110.63, 87.52, 83.41, 82.07, 78.78, 77.21, 70.35, 68.28, 62.17, 58.64, 58.34, 58.14, 55.29, 55.24, 45.45, 42.55, 12.40, 11.05; MS (electrospray$^+$) m/e 872 (M + H$^+$), 894 (M + Na$^+$); MS (electrospray$^-$) m/e 870 (M − H$^-$), 906 (M + Cl$^-$). Continued elution of the column provided a substantial portion of mixed fractions, followed by pure **20b**: R_f 0.12 (2% MeOH in 4:1 EtOAc−hexane); ^{1}H NMR ($CDCl_3$) δ 9.11 (s, 1H), 9.06 (s, 1H), 7.85 (s, 1H), 7.47 (s, 1H), 7.44 (d, 2H), 7.35−7.20 (m, 7H), 6.82

(m, 4H), 5.89 (s, 1H), 5.81 (d, J = 1.7, 1H), 4.16 (d, 1H), 4.03 (dd, 1H), 3.92 (m, 3H), 3.79 (s, 6H), 3.75 (m, 3H), 3.59 (s, 3H), 3.58 (s, 3H), 3.28 (d, 1H), 3.00 (m, 1H), 2.71 (m, 1H), 2.57 (s+m, 3H+1H), 1.88 (s, 3H), 1.36 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 164.19, 163.65, 158.66, 150.22, 150.12, 144.27, 135.53, 135.46, 135.38, 130.17, 128.27, 127.92, 127.13, 113.16, 110.57, 110.22, 88.97, 87.76, 86.59, 86.00, 83.62, 83.30, 82.05, 77.21, 68.60, 62.54, 58.64, 58.21, 56.18, 55.24, 45.74, 39.01, 12.45, 11.92; MS (electrospray$^+$) m/e 872 (M + H$^+$), 894 (M + Na$^+$); MS (electrospray$^-$) m/e 870 (M − H$^-$), 906 (M + Cl$^-$).

3′-De(oxyphosphinico)-3′-[methylene(methylimino)]-5′-*O*-(4,4′-dimethoxytriphenylmethyl)-2-*N*-isobutyryl-2′-*O*-methylguanosylyl-(3′→5′)-3′-*O*-(*tert*-butyldiphenylsilyl)-2′-*O*-methyl-5-methyluridine (19d). Compound **19c** (348 mg, 0.38 mmol) was reacted with DMTCl (350 mg, 1.03 mmol) in the presence of DMAP (30 mg) in a manner similar to that described for the preparation of **20a** and **20b**, and the product was purified by chromatography (5 to 7% MeOH/CH_2Cl_2) to provide 345 mg (74%) of **19d**: ^{1}H NMR ($CDCl_3$) δ 12.08 (bs, 1H), 11.60 (bs, 1H), 11.38 (bs, 1H), 8.03 (s, 1H), 7.62−7.08 (m, 21H), 6.80 (d, 4H), 6.00 (s, 1H), 5.78 (s, 1H), 4.20 (bs, 1H), 4.12−3.95 (m,3H), 3.76 (s, 6H), 3.50−2.58 (m, 19H), 2.25 (s, 3H), 1.60 (s, 3H), 1.20−1.15 (m, 6H), 1.00 (s, 9H); MS (FAB$^+$) m/e 1206 (M + H). Anal. Calcd for $C_{65}H_{76}N_8O_{13}Si\cdot 0.5H_2O$: C, 64.29; H, 6.39; N, 9.23. Found: C, 64.25; H, 6.41; N, 9.02.

3′-De(oxyphosphinico)-3′-[methylene(methylimino)]-2′-*O*-methylguanosylyl-(3′→5′)-2′-*O*-methyl-5-methyluridine (19e). A solution of **19d** (140 mg, 0.12 mmol) in dioxane (2 mL) plus NH_4OH (37%, 2 mL) was heated at 55 °C in a sealed tube for 15 h. The solvent was removed, and the residue was azeotroped with MeCN (3 × 10 mL) and then dissolved in THF (2 mL) and treated with Bu_4NF on silica gel (0.32 g, contains 0.35 mmol of Bu_4NF) with stirring for 20 h at rt. The product was chromatographed (60:20:10 EtOAc/hexane/MeOH and then 10% MeOH/CH_2Cl_2), and the resulting product dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (3 mL) and vigorously stirred for 3 h. MeOH (0.5 mL) was added, the solvent removed, and the residue chromatographed (7−12% MeOH/CH_2Cl_2) to provide 38 mg (77%) of **19e** as a colorless foam: ^{1}H NMR (DMSO-d_6) δ 8.00 (s, 1H), 7.52 (s, 1H), 6.38 (bs, 2H), 5.83 (s, 1H), 5.80 (s, 1H), 5.14 (bs, 1H), 5.00 (bs, 1H), 4.07−3.50 (m, 9H), 3.40 (s, 3H), 3.25 (s, 3H), 2.97 (m, 1H), 2.65 (m, 1H), 2.58 (s, 3H), 1.80 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 163.65, 155.58, 153.62, 150.47, 150.33, 135.77, 134.75, 116.67, 109.65, 86.39, 85.98, 85.85, 84.48, 82.28, 81.71, 71.20, 68.76, 61.15, 57.77, 57.34, 56.57, 45.50, 12.13. Anal. Calcd for $C_{24}H_{34}N_8O_{10}\cdot 0.25H_2O$: C, 46.62; H, 5.73; N, 17.93. Found: C, 46.48; H, 5.37; N, 17.91.

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Supporting Information Available: Full synthetic details and selected data for compounds **6c**, **7a**−**c**, **9a**−**c**, **9e**, **10c**, **10e**, **10f**, **11a**−**k**, **12b**, **12d**, **12h**, **12i**, **14a**, **14b**, **15a**−**d**, **16a**−**g**, **16i**−**l**, and **18** (21 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions.

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