Synthesis and Conformational Properties of 2'-Deoxy-2'-methylthio-pyrimidine and -purine Nucleosides: Potential Antisense Applications

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Dedicated to the memory of Dr. Roland K. Robins

A convenient and shorter synthesis of 2'-deoxy-2'-methylthiouridine analogs **5**, -5-methyluridine **6**, -cytidine **15**, -5-methylcytidine **16**, -adenosine **27** and -guanosine **34** was accomplished. Successful conversion of ribonucleosides (5-methyl U, U, A, G) into the corresponding 2'-substituted nucleosides involves nucleophilic displacement (S_N2) of an appropriate leaving group at the 2'-position by methanethiol, a soft nucleophile. Reaction between 2,2'-anhydrouridine and methanethiol in the presence of N^1,N^1,N^3,N^3-tetramethylguanidine in N,N-dimethylformamide gave **5** in 75% yield. Preparation of **6** by a similar route was described. Acylated **5** and **6** were transformed into their triazole derivatives, which on ammonolysis furnished **15** and **16**, respectively in good yield. Similarly, tetraisopropyldisiloxanyl (TIPS) protected 2'-O-aratriflates- of -adenosine and -guanosine reacted with methanethiol in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene at −25°, followed by deblocking of the TIPS protecting group furnished **27** and **34**, respectively. The conformational flexibility (N/S equilibrium) of the sugar moiety in nucleosides **5**, **15**, **27** and **34** was studied utilizing nmr spectroscopy, suggesting that the 2'-methylthio group influenced the sugar conformation to adopt a rigid S-pucker in all cases. The extra stiffness of the sugar moiety in these analogs is believed to be due to the electronegativity of the substituent and the steric bulk. The usefulness of these nucleosides to prepare uniformly modified 2'-deoxy-2'-methylthio oligonucleotides for antisense therapeutics is proposed.

Introduction.

Antisense oligonucleotides (AO) and their analogs are of interest as potential drugs that would modulate gene expression as a mode of action [1]. The 2'-functional group modifications have been utilized extensively as one of the class of sugar modified AO [2]. The interest in 2'-sugar modification for AO is based on: (a) synthetic access to nucleoside analogs and their incorporations into AO *via* automated synthesis [3], (b) formation of more stable duplexes [4] and triplexes [5] with complementary nucleic acids, (c) enhanced stability against degradation by cellular nucleases [6], (d) opportunity to study the distribution of N/S puckering which distinguishes RNA from DNA [7], and (e) application in nmr studies of duplexes [8].

The effects of stereochemistry and electronegativity of the 2'-substituents and their role in sugar conformation at the oligomer level is of significant interest, due to altered binding and biological properties of AO. Various studies with modified nucleosides and oligonucleotides have established that the furanyl-ring adopts one of two principal puckered shapes [9], which are 3'-endo (N) and 2'-endo (S) (Figure I). The two conformations characterize two distinctly different families (A and B) of nucleic acids structure and produce a variety of duplex models. According-

ing to AO studies published to date, appropriate 2'-substitution in an oligomer allowed a high N-pucker of the sugar ring, which accounts for improved binding to target RNA [10]. A recent study by Kawasaki *et al.* [11] utilized a highly electronegative fluorine substituent, which is known to have high population of N-pucker, to construct a uniformly modified AO. The study suggested that uniform incorporation of 2'-deoxy-2'-fluoronucleosides in an oligomer provided a better mimic of RNA, when compared to unmodified RNA. In contrast, an improved mimic of DNA, which exhibits a high S-pucker has not been investigated thus far. A literature search revealed that 2'-thionucleosides preferred a high 2'-endo (80% S-type) conformation [12], which is much greater than 2'-deoxynucleoside (65% S-type). In 1975, Imazawa *et al.* expressed the desire to study the effects of a 2'-thio substitutent on conformational properties at the nucleoside and oligonucleotide level, which has not been fulfilled to this date.

In the past decade numerous thio sugars and their nucleoside derivatives have been prepared for their biological properties [13]. Among these analogs, 2'-deoxy-2'-methylthionucleosides appear to be the analog of choice for incorporations into AO for several reasons. First, there has been literature precedent to the chemical routes for the introduction of a 2'-alkyl/arylthio group into ribonucleosides [14]. Secondly, the bulky methylthio group at the 2'-position of the sugar may prefer a *quasi*-equatorial orientation in the C2'-endo mode (S-pucker), over a *quasi*-axial orientation (N-pucker). Thirdly, is the ease of incorporation of 2'-modified nucleosides into AO *via* automated DNA synthesizer. Lastly, is the use of the 2'-methylthio
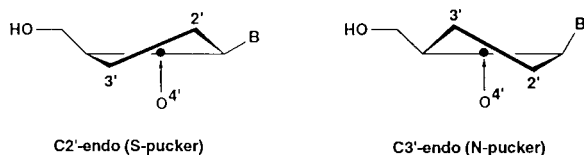
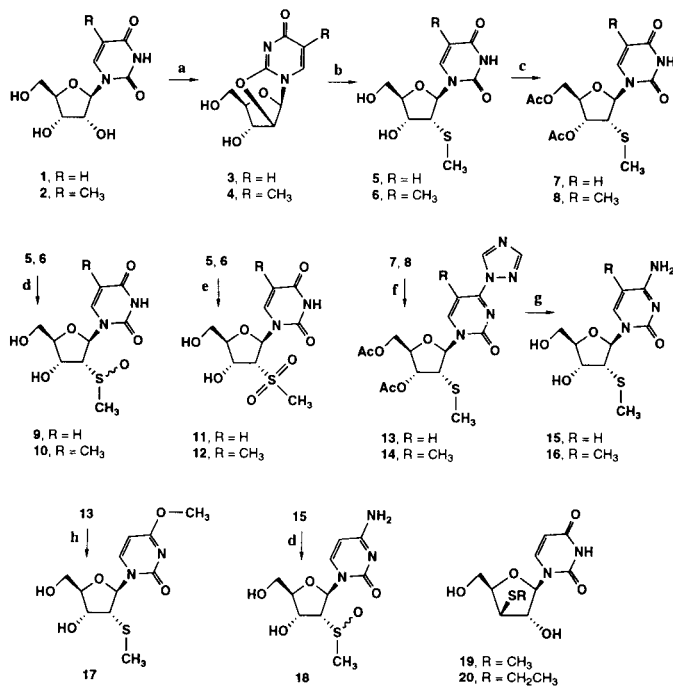C2'-endo (S-pucker)   C3'-endo (N-pucker)

Table I

Preparation and Physical Properties of Nucleosides

Starting Material	Product	Method of Preparation	Yield %	Melting Point (°C)	Molecular Formula	Elemental Analysis							
						Calculated				Observed			
						C	H	N	S	C	H	N	S
3	5	b	75	112-114 [a]	$C_{10}H_{14}N_2O_5S$	43.79	5.14	10.21	11.69	43.98	5.14	10.16	11.6
4	6	b	74	172-173	$C_{11}H_{16}N_2O_5S$	45.82	5.59	9.72	11.12	45.6	5.63	9.73	10.73
5	7	c	77	140-142	$C_{14}H_{18}N_2O_7S$ [b]	47.18	5.46	7.43	8.51	47.57	5.12	7.66	8.69
5	9	d	48	198-200	$C_{10}H_{14}N_2O_6S$	41.38	4.86	9.65	11.05	41.37	4.87	9.52	11.08
5	11	e	68	227-228	$C_{10}H_{14}N_2O_7S$	39.21	4.59	9.15	10.47	39.3	4.61	9.00	10.45
5	8	c	75	143-144	$C_{15}H_{20}N_2O_7S$	48.38	5.41	7.51	8.61	48.42	5.44	7.46	8.57
6	10	d	42	197-198	$C_{11}H_{16}N_2O_6S$	43.42	5.3	9.21	10.53	43.6	5.53	8.83	10.27
6	12	e	36	117-118	$C_{11}H_{16}N_2O_7S$	41.25	5.04	8.75	10.01	41	5.19	8.56	9.73
7	13	f	64	124-125	$C_{16}H_{19}N_5O_6S$	46.94	4.68	17.11	7.85	46.63	4.61	17.07	7.68
8	14	f	64	210-211	$C_{17}H_{21}N_5O_6S$	48.22	4.99	16.54	7.57	48.17	4.97	16.37	7.60
13	15	g	86	189-190	$C_{10}H_{15}N_3O_4S$ [c]	43.23	5.62	15.12	-	43.26	5.43	14.73	-
13	17	h	23	155-156	$C_{11}H_{16}N_2O_5S$	45.83	5.59	9.72	-	45.75	5.64	9.66	-
14	16	g	99	122-124	$C_{11}H_{17}N_3O_4S$	45.98	5.96	14.62	11.16	45.70	6.12	14.26	11.09
15	18	d	75	150-152	$C_{10}H_{15}N_3O_5S$	41.52	5.23	14.52	11.08	41.51	5.18	14.16	10.96
21	22	Ref. [13a]	86	foam	$C_{23}H_{41}N_5O_7Si_2S$	47.00	7.03	11.91	5.45	47.21	7.05	11.62	5.37
22	25	i	50	230 dec.	$C_{11}H_{15}N_5O_3S$	44.44	5.09	23.55	10.78	44.17	4.99	23.27	10.85
23	26	j	95	151-152	$C_{23}H_{41}N_5O_4SSi_2$	51.17	7.66	12.97	5.94	51.25	7.67	12.79	5.79
23	28	k	99	149-151	$C_{23}H_{37}N_5O_4Si_2$	53.73	7.59	14.24	-	53.59	7.41	13.81	-
23	33	j	74	230 dec.	$C_{23}H_{41}N_5O_5SSi_2$ [c]	49.30	7.47	12.50	5.72	49.16	7.37	12.15	5.69
26	27	l	93	172-173	$C_{11}H_{15}N_5O_3S$	44.44	5.09	23.55	10.78	44.39	4.95	23.26	10.54
28	29	l	93	224-225	$C_{10}H_{11}N_5O_3$	48.19	4.45	28.10	-	48.11	4.32	27.80	-
33	34	m	33	210	$C_{11}H_{15}N_5O_4S$ [d]	37.93	4.85	20.10	9.20	37.73	4.70	20.45	9.39

[a] 124-126° in reference 12. [b] Calculated with 0.4 mole of EtOH. [c] Calculated with 0.25 mole of H_2O. [d] Calculated with 1.75 moles of HF.

Scheme I

Reaction conditions: Please refer to experimental section for the details of methods a-h

Table II

UV Spectral Data of Nucleosides in Methanol

Compound	UV Max (nm)	ε
5	262	9,648
6	268	10,110
9	258	10,273
10	264	10,406
11	258	10,228
12	264	11,576
15	272	7,415
16	280	6,590
17	278	5,196
18	270	7,062
25	260	15,118
27	260	15,283
29	252	17,518
34	256	13,186

singlet in ¹H nmr as an internal standard for the character-ization of modified oligonucleotides. However, in order to synthesize such oligonucleotides, an efficient procedure for the large-scale synthesis of 2'-methylthionucleosides must be available.

The main routes to such derivatives have been either a coupling of thiosugars with heterocyclic bases, as in the synthesis of a 2'-deoxy-2'-methylthioadenosine derivative by Ryan et al. [13a], or by nucleophilic S$_N$2 opening of the 2,2'-anhydrouridine as described by Imazawa et al. [12]. A more convenient method of direct introduction of a 2'-α-thio substituent into preformed purine and pyrimidine nucleosides has been reported by Divakar et al. [14a,c]. In this paper we describe an improved version of Divakar's procedure to prepare 2'-deoxy-2'-methylthio- analogs of -uridine **5**, -5-methyluridine **6**, -adenosine **27** and -guanosine **34** in good yields. The conformational proper-ties of these nucleosides were also investigated by ¹H nmr spectroscopy.

Results and Discussion.

The synthesis of pyrimidine analogs is illustrated in Scheme I. Crystalline 2,2'-anhydrouridine **3** was readily prepared from uridine **1** by a published procedure in one step [15]. Cleavage of **3** has been studied with various nucleophiles, including alkyl- and aryl-thiolate ions [14]. Based on these results, nucleoside **3** was heated with ~4.8

molar equivalents of methanethiol, 5 molar equivalents of N^1,N^1,N^3,N^3-tetramethylguanidine in N,N-dimethylform-amide (DMF) solution for 12 hours at 60°, thus 2'-deoxy-2-methylthiouridine **5** was obtained in 75% yield as the only nucleoside product (Table I). In a similar manner, **4** was transformed to **6** in 74% isolated yield. The latter com-pounds **5** and **6** were characterized on the basis of their elemental analysis and spectral data (Tables I-III, V). The ¹H nmr spectra of **5** and **6** had H-2' peak shifts (δ 3.37) up-field relative to those of starting materials **3** and **4** (δ 5.19 and 5.18, respectively). The $J_{1',2'}$ = 8.5 and 8.68 Hz, cou-pling constants for **5** and **6**, respectively, are indicative of trans configurations and are in agreement with literature values [14a,b]. However, Brown et al. [16] reported that treatment of **3** with sodium ethanethiolate in DMF led to the formation of **20**. In view of Brown's observation, the possibility of formation of xylonucleoside **19** was ruled out for several reasons. Treatment of weakly basic soft nucleo-phile such as methanethiol on **3** should not give the unde-sired nucleoside **19**, under the described conditions. Furthermore, our results are in agreement with those of Robins [13d], Divakar [14a,c], Imazawa [12] and Matsuda [14b] reporting direct substitution having occurred to give the expected ribo-configuration at C-2', under similar con-dition as we report herein. Finally, our sample of **5** had similar properties with that reported in the literature [12]. Treatment of **5** and **6** with acetic anhydride in pyridine at ambient temperature gave the acylated **7** and **8**, respec-tively. The protected **7** and **8** were converted to their cyti-dine counterparts **15** and **16**, respectively by a reported procedure [17]. Thus, treatment of **7** and **8** with phos-phorus oxychloride/pyridine/1,2,4-triazole gave the 4-(1,2,4-triazol-1-yl)pyrimidin-2-one derivatives **13** (64%) and **14** (64%), respectively. The latter compounds were treated with ammonium hydroxide/1,4-dioxane to give **15** (86%) and **16** (95%), respectively. However, in another experiment, treatment of **13** in saturated (0°) methanolic

Table III

^{1}H NMR Shifts of Pyrimidine Analogs in Dimethyl Sulfoxide-d_6 (ppm)

Compound	H-1'	H-2'	H-3'	H-4'	H-5',5"	H-5	H-6	N(H)	NH$_2$	CH$_3$	S(O)$_n$CH$_3$	COCH$_3$	OH-3'	OH-5'
4	6.30 (d)	5.18 (d)	4.39 (b,s)	4.08 (m)	3.25 (m)	-	7.76 (s)	-	-	1.81 (s)	-	-	5.89 (d)	4.98 (t)
5	6.03 (d)	3.37 (d,d)	4.19 (m)	3.84 (d,d)	3.54 (b,d)	5.68 (d)	7.84 (d)	11.37 (b,s)	-	-	1.99 (s)	-	5.63 (d)	5.10 (t)
6	6.03 (d)	3.37 (d,d)	4.18 (m)	3.83 (d,d)	3.53 (b,d)	-	7.69 (d)	11.35 (s)	-	1.77 (s)	1.99 (s)	-	5.58 (d)	5.10 (t)
7	6.10 (d)	3.38 (d,d)	5.29 (d,d)	4.33 (m)	4.33 (m)	5.80 (d)	7.44 (d)	9.34 (b,s)	-	-	2.14 (s)	2.17 (s)	-	-
8	6.12 (d)	3.37 (d,d)	5.30 (d,d)	4.33 (m)	4.33 (m)	-	7.21 (d)	9.28 (s)	-	1.95 (d)	2.15 (s)	2.19 (s)	-	-
9	6.29 (d)	3.92 (d)	4.57 (b,s)	3.79 (d,d)	3.56 (b,d)	5.72 (d)	7.89 (d)	11.47 (b,s)	-	-	2.52 (s)	-	6.14 (d)	5.20 (b,d)
10 [a]	6.28 (d)	3.79 (d,d)	4.56 (m)	3.90 (b,t)	3.57 (b,t)	-	7.75 (d)	11.42 (s)	-	1.77 (d)	2.50 (s)	-	6.13 (d)	5.21 (t)
10 [b]	6.56 (d)	3.67 (m)	4.56 (d,d)	3.67 (m)	3.67 (m)	-	7.63 (d)	11.39 (s)	-	1.80 (d)	2.60 (s)	-	6.04 (d)	5.05 (t)
11	6.49 (d)	3.94 (d)	4.49 (m)	3.99 (m)	3.57 (m)	5.73 (d)	7.78 (d)	11.45 (s)	-	-	3.13 (s)	-	6.30 (d)	5.20 (b,s)
12	6.48 (d)	4.00 (m)	4.50 (b,d)	3.95 (m)	3.57 (b,d)	-	7.63 (d)	11.43 (s)	-	1.77 (d)	3.12 (s)	-	6.29 (d)	5.21 (t)
13 [c,e]	6.23 (d)	3.69 (d,d)	5.16 (t)	4.45 (m)	4.45 (m)	7.09 (d)	8.30 (d)	-	-	-	2.32 (s)	2.17 (s)	-	-
14 [c,f]	6.23 (d)	3.67 (d,d)	5.18 (t)	4.47 (m)	4.44 (m)	-	8.02 (s)	-	-	2.31 (s)	2.50 (s)	2.17 (s)	-	-
15	6.10 (d)	3.27 (d,d)	4.17 (m)	3.81 (m)	3.54 (b,s)	5.73 (d)	7.77 (d)	-	7.24 (b,s)	-	1.96 (s)	-	5.55 (d)	5.06 (t)
16	6.08 (d)	3.31 (d,d)	4.18 (m)	3.80 (m)	3.55 (m)	-	7.60 (s)	-	7.38, 6.87 (d)	1.82 (s)	1.96 (s)	-	5.50 (d)	5.06 (t)
17 [d]	6.16 (d)	3.37 (m)	4.26 (m)	3.92 (m)	3.60 (m)	6.12 (d)	8.24 (d)	-	-	-	2.03 (s)	-	5.64 (d)	5.18 (t)
18	6.55 (d)	3.42 (d,d)	4.51 (m)	3.70 (m)	3.62 ,3.52 (m)	5.73 (d)	7.68 (d)	-	7.23 (s)	-	2.57 (s)	-	5.98 (d)	4.94 (t)

[a] High R$_f$ isomer. [b] Low R$_f$ isomer. [c] Deuteriochloroform. [d] OCH_3 at 3.85 (s). [e] Triazole H at 8.13 (s) and 9.12 (s). [f] Triazole H at 8.13 (s) and 9.12 (s).

Table IV

^{1}H NMR Shifts of Purine Analogs in Dimethyl Sulfoxide-d$_6$ (ppm)

Compound	H-1'	H-2'	H-3'	H-4'	H-5',5"	H-2	H-8	NH	NH$_2$	3'-OH	5'-OH	SCH$_3$	TIPS
22 [a]	6.46 (d)	5.63 (t)	5.35 (t)	4.23 (m)	3.94 (m)	8.09 (s)	8.17 (s)	-	7.39 (b,s)	-	-	-	1.05 (m)
25 [b]	5.73 (d)	4.67 (d,d)	3.35 (d,d)	4.37 (m)	3.67 (b,t)	8.13 (s)	8.32 (s)	-	7.37 (b,s)	-	5.55 (t)	2.18 (s)	-
26	6.13 (d)	4.15 (d,d)	5.05 (m)	3.32 (b,s)	3.97 (b,s)	8.10 (s)	8.33 (s)	-	7.35 (b,s)	-	-	2.00 (s)	1.05 (b,m)
27	6.12 (d)	4.36 (b,t)	4.03 (m)	4.03 (m)	3.65 (m)	8.17 (s)	8.44 (s)	-	7.41	5.73 (d)	5.41 (t)	1.77 (s)	-
28	-	5.74 (d)	5.5 (t)	4.58 (m)	3.90 (m) 4.21 (d,d)	8.28 (s)	8.30 (s)	-	7.54 (b,s)	-	-	-	1.05 (b,s)
29	-	5.68 (d)	4.83 (m)	4.41 (m)	3.58 (b,t)	8.28 (s)	8.34 (s)	-	7.50 (b,s)	5.37 (d)	5.03 (t)	-	-
32 [c]	6.15 (d)	5.33 (t)	4.74 (t)	3.87 (m)	4.06 (b,s)	-	7.66 (s)	12.06 (b,s)	6.88 (b,s)	-	-	-	1.05 (b,m)
33 [c]	5.97 (d)	4.10 (m)	4.78 (t)	3.64 (m)	4.10 (m)	-	7.77 (s)	12.07 (b,s)	6.47 (b,s)	-	-	2.18 (s)	1.05 (b,m)
34	5.89 (d)	3.80 (d,d)	4.26 (b,t)	3.87 (b,t)	3.53 (b,d)	-	7.97 (s)	10.67 (b,s)	6.49 (b,s)	5.63 (d)	5.04 (t)	1.81 (s)	-

[a] OMS at 3.0 (s). [b] 2'-OH at 5.92 (d). [c] In deuteriochloroform.

Table V

^{13}C NMR Shifts of Pyrimidine Analogs in Dimethyl Sulfoxide-d$_6$ (ppm)

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-2	C-4	C-5	C-6	C-5 CH$_3$	S(O)$_n$CH$_3$	COCH$_3$	Triazole	COCH$_3$
4	89.1	90.2	74.7	88.5	60.9	171.7	159.4	116.7	132.3	13.6	-	-	-	-
5	87.4	52.8	72.0	86.5	61.4	150.7	162.9	102.3	140.3	-	14.0	-	-	-
6	87.0	52.0	72.0	86.1	61.0	150.4	163.5	110.0	138.0	13.9	12.1	-	-	-
7	87.2	49.4	73.4	80.3	63.4	150.6	162.8	103.0	140.2	-	20.6	14.3	-	170.2, 169.5
8	86.9	49.2	73.5	80.2	63.4	150.7	163.5	110.6	135.6	12.2	20.6	14.2	-	168.6, 170.2
9	82.2	87.1	71.7	67.8	61.3	150.6	162.7	102.9	140.7	-	36.0	-	-	-
10	82.1	87.1	71.7	67.9	61.4	150.8	163.7	110.9	136.4	12.4	36.1	-	-	-
11	82.1	86.5	70.7	67.8	60.9	150.4	162.8	102.8	140.3	-	41.8	-	-	-
12	81.9	86.4	70.7	67.6	60.9	150.5	163.5	110.4	135.7	12.3	41.8	-	-	-
13	89.9	51.1	73.0	80.9	63.2	159.0	153.7	95.2	148.6	-	20.6	14.3	144.0, 154.3	169.6, 170.3
14	89.5	50.8	73.1	80.9	63.1	158.5	153.2	105.9	148.0	16.2	20.6	14.2	145.6, 153.6	169.5, 170.2
15	88.3	53.4	71.8	86.0	61.4	155.3	165.5	94.6	141.3	-	13.7	-	-	-
16	88.1	53.1	71.7	85.8	61.3	155.1	165.1	101.7	138.3	13.6	13.3	-	-	-
17[a]	88.6	53.9	71.3	86.4	61.0	154.9	171.1	95.4	144.3	-	13.7	-	-	-
18	82.4	84.5	69.2	67.2	60.1	154.6	165.5	94.9	142.3	-	36.1	-	-	-

[a] O^4-CH$_3$ at 54.26.

ammonia resulted in formation of a relatively nonpolar by-product **17** (23%) in addition to desired **15** (9%). The uv and ^{1}H nmr spectral data (Table II, III) were compatible with the formation of O^4-methyl derivative **17**. Analogous reactions have been reported [18] in the literature where

solvent acts as a nucleophile displacing the triazole group.

The standard automated synthesis of oligonucleotides requires an oxidation step [19] during the synthesis utilizing various oxidation reagents. We planed to utilize the standard protocol to incorporate the described 2'-methyl-

Table VI

^{13}C Shifts of Purine Analogs in Dimethylsulfoxide-d$_6$ (ppm)

Compound	C-1'	C-2'	C-3'	C-4'	C-5'	C-2	C-4	C-5	C-6	C-8	SCH$_3$
25	80.70	52.46	77.93	88.32	61.89	152.45	149.08	119.29	156.23	139.98	15.59
27	87.48	52.85	72.41	88.50	61.80	152.58	149.12	119.28	156.18	139.82	13.70
29	145.76	88.17	73.62	89.82	61.29	153.68	148.18	119.09	156.23	136.82	-
34	86.89	53.03	57.51	72.14	61.57	153.67	151.39	116.49	156.74	135.69	13.55

thionucleosides into various oligonucleotides. Therefore, one of the obvious concerns we had was the oxidation of sulfide-nucleosides described in this paper, under standard conditions. In view of this, we prepared the sulfoxide and sulfone derivatives of **5**, **6**, and **15** as our reference samples.

Controlled oxidation of **5**, **6**, and **15** to their diastereomeric sulfoxides **9** (61%), **10** (48%) and **18** (75%), respectively, was achieved by using 1.0 molar equivalent of 3-chloroperoxybenzoic acid in ethanol at 0°. One diastereomeric sulfoxide **10** was separated (see Table III for nmr data) by chromatography, but the conformation at the sulfur was not determined. The modest yield of selective oxidation was further decreased by the troublesome isolation of the sulfoxides by chromatography. Complete oxidation of sulfides **5** and **6** to the corresponding sulfones **11** (68%) and **12** (36%) was carried out with 4 molar equivalents of 3-chloroperoxybenzoic acid in ethanol at ambient temper-

Scheme II

21, R = H
22, R = SO$_2$CH$_3$
23, R = SO$_2$CF$_3$

24

25

26, R = R = -TIPS-
27, R = R = H

28, R = R = -TIPS-
29, R = R = H

30

[Ref. 25] 31, R = H
32, R = SO$_2$CF$_3$

33

34

Reaction conditions: Please refer to experimental section for the details of methods a-m

ature. The ^{1}H nmr of these compounds showed characteristic downfield shifts of the methylthio group by ~0.56 ppm for the sulfoxides and ~1.14 ppm for the sulfones, relative to their parent sulfides (Table III). In addition, the ^{13}C nmr exhibited large downfield shifts of the C-2' absorption for sulfoxides and sulfones, compared to their parent sulfides (Table V), consistent with observation by Mansuri et al. [20] for C-3' thionucleosides. Carbon assignments in Table V were achieved, in part, through the use of heteronuclear multiple quantum correlation experiments (HMQC). The sulfides **6**, **15**, and **16**, sulfoxides **9**, **10**, and **18**, and sulfones **11** and **12** represents a new series of 2'-substituted thiopyrimidine analogs that may potentially have biological activity [21].

The synthesis of purine analogs is illustrated in Scheme II. In order to synthesize the 2'-deoxy-2'-methylthioadenosine (**27**) we used commercial [22] 9-β-D-arabinofuranosyladenine, which was easily converted to the protected nucleoside 9-[3,5-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]adenine (**21**) via a literature procedure [23]. The latter compound was converted to its 2'-O-mesyl derivative **22** following a standard procedure [13a]. Inspired by the clean displacement reactions demonstrated by Mansuri et al. [20] and Ryan et al. [13a] with sodium methylmercaptide, we decided to employ the latter reagent to effect the S$_N$2 displacement of 2'-O-mesyl group of **22** in dry dimethylformamide. In 12 hours a crystalline polar product (76%) was formed. The sole nucleoside product obtained from this reaction had an expected adenosine type uv spectrum but different ^{1}H and ^{13}C nmr spectra, compared to the 2'-thioadenosine described by Mariott et al. [23]. Complete assignment of each proton resonance (see Tables IV, VI) in this polar nucleoside was carried out by TOCSY and NOESY techniques, proposing a xylo configuration for 3'-methylthio group, as depicted in structure **25**. We rationalize the formation of 9-[3-deoxy-3-(methylthio)-β-D-xylofuranosyl]adenine **25** in the following manner. Under basic conditions, first, the tetraisopropyldisiloxanyl protecting group of **22** was deblocked and then 2'-O-mesylate was trans-eliminated to yield an intermediate epoxide **24**, which then undergoes nucleophilic β-phase attack by sodium methanethiolate ion at C-3' position to give nucleoside **25**. This hypothesis is in good agreement with the reported literature [24].
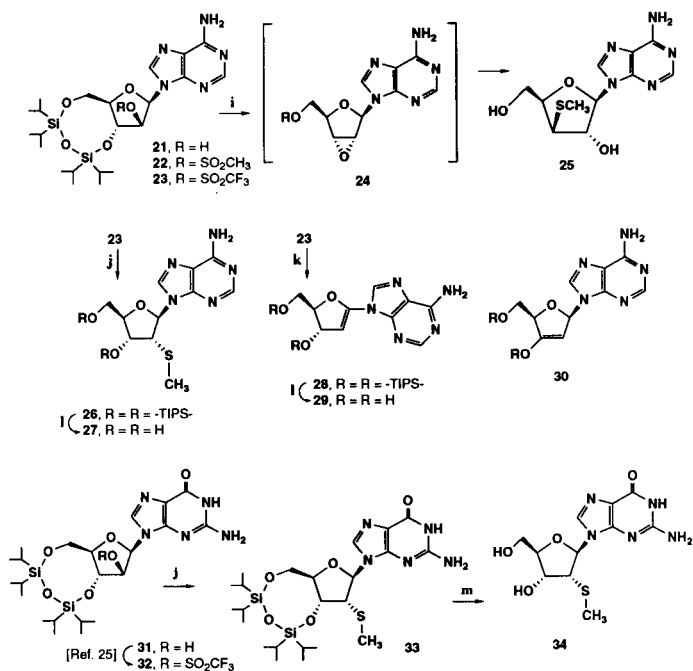
Table VII

Variable Temperature ^{1}H nmr Studies of Nucleosides 5, 15, 27 and 34 in CD3OD

Compound	Temperature °C	H1',2'	J (Hz) H3',4'	% S
5	20	8.2	2.57	76
	0	8.2	2.48	77
	-20	8.29	2.31	78
	-40	8.32	2.21	79
15	20	7.65	3.24	70
	0	7.64	3.24	70
	-20	7.87	3.01	72
	-40	7.87	2.78	74
27	20	9.01	1.07	89
	0	9.16	0.61	93
	-20	9.16	0	100
	-40	9.31	0	100
34	20	8.85	1.68	84
	0	8.85	1.52	85
	-20	9.0	1.22	88
	-40	9.01	0.92	91

In the search for an appropriate method for the synthesis of desired 27, a recent report by Robins et al. [25] appeared to be useful. This report described an efficient displacement of a 2'-O-triflate group from the purine nucleoside by an azide nucleophile. Therefore, 2'-O-triflate 23 was prepared following the published procedure. The reaction between the triflate 23 and methanethiol proceeded rapidly in DMF in the presence of a strong hindered base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) to furnish a mixture (1:1.2) of two products (70%). The two products were easily separated by chromatography and characterized as the desired 2'-methylthio derivative 24 and an elimination product. Formation of an elimination product utilizing DBU is well documented in the literature [26]. Deblocking of 26 with tetrabutylammonium fluoride afforded the expected nucleoside 27 in 93% yield. In a similar way, the elimination product 28 was deblocked to furnish a crystalline compound. We propose the structure of the latter compound to be 29, which is based on its elemental analysis, nmr and mass spectral data. Formation of an alternative elimination product 30 was ruled out on the basis of literature precedent for very similar reactions [27]. However, this S$_N$2 displacement reaction resulted in a low yield (32%) of the desired product, partially due to the competing elimination of the 2'-O-triflate group. One of the possibility of favored elimination in nucleoside 23 may be due to the trans-diaxial disposition of the anomeric proton and C-2' leaving group, thus, making the formation of 28 facile. Therefore, modification of the reaction conditions of the present route was warranted. All attempts to employ a weaker base such as triethylamine and diisopropylethylamine for the conversion of 23 to 26 at room temperature and at 50° resulted in no reaction and starting material was recovered. Treatment of 23 with N^1,N^1,N^3,N^3-tetramethylguanidine at 50° resulted in the formation of 28. After several attempts and fine tuning of the original ex-

periment with DBU, the following conditions were developed for best results. Triflate 23 was converted smoothly into desired nucleoside 26 in 95% yield, upon treatment with 3 molar equivalents of methanethiol and 4 molar equivalents of DBU in dry DMF at −25° for 1 hour. No elimination product was detected under the described conditions. We found, however, that the elimination product 28 could be prepared very effectively in 99% yield, when 23 was treated with methanethiol/DBU in dichloromethane at ambient temperature for 1 hour.

Having optimized S$_N$2 displacement conditions in the presence of DBU for the preparation of the adenosine analog 27, the synthesis of 2'-deoxy-2'-methylthioguanosine appeared to be straightforward. We prepared 9-[3,5-O-(1,1,3,3-tetra-isopropyldisiloxane-1,3-diyl)-β-D-arabinofuranosyl]guanine 31 utilizing a modified procedure described by Kawasaki et al. [11], as our starting material. The tetraisopropyldisiloxanyl protected 31 was triflated efficiently (trifluoromethanesulfonyl chloride/4-dimethylaminopyridine) in dichloromethane to give 2'-O-triflate 32 (75%) (Scheme II). The ara-triflate 32 underwent nucleophilic substitution (methanethiol/DBU/dimethylformamide) smoothly at −25° to give the 2'-methylthio derivative 33 in high yields (75%) without formation of an elimination product. The tetraisopropyldisiloxanyl protected 33 was deblocked (hydrogen fluoride-pyridine/tetrahydrofuran) to give the desired nucleoside 34 in modest yields (33%). The ^{1}H and ^{13}C nmr and uv spectra confirmed the assigned structure of 34 (Tables II, IV, VI). The elemental analysis of the latter sample indicated that it was cocrystallized with hydrogen fluoride.

Conformation Studies.

The pentofuranosyl moiety is the essential link that connects the negatively charged phosphates and heterocyclic bases into the unique chemical framework of an oligonucleotide, such as DNA or RNA. The inherent conformational flexibility of the sugar moiety is believed to have an important bearing on the structure of oligonucleotides and their biological functions. The pentofuranosyl rings are always twisted out of plane in order to minimize nonbonded interactions between their substituents. This puckering mode is described by the two basic forms: C3'-endo (N) and C2'-endo (S) (Figure I). Proton nmr spectroscopy has been used by several investigators to study and calculate the percentage of N- and S-pucker in solution adopted by various nucleosides [7,28]. It is believed that the sugar conformations in oligonucleotides are closely related to those predicted in the corresponding properties of nucleosides [28a]. In fact, the position and orientation of the 2'-substituents have a definite and predictable influence on the conformation of oligonucleotides, which subsequently affects the hybridization properties, a key feature for the design of AO.

We elected a recently reported [7] variable temperature ^{1}H nmr technique to calculate the N/S equilibria for nucleosides **5, 15, 27** and **34**, using deuteriomethanol as a solvent. The results indicated that the ^{1}H nmr spectra of nucleosides studied do not vary significantly over the temperature range $-40°$ to $20°$ (Table VII). The puckering equilibrium of the sugar ring was analyzed by the use of vicinal spin-coupling constants, using the formula: % S = 100 x J 1',2'/(J 1',2' + J 3',4'). The findings in Table VII suggested that the conformation of the sugar ring in pyrimidine analogs shows an average of >75% S-character, whereas, the purine analogs exhibits an average of >91% S-pucker. It is evident from the results, that all compounds studied here, adopted a rigid geometry with a high S-pucker, compared to 65% (S) for a 2'-deoxynucleoside. The characteristic peaks in the nmr spectra of these compounds **5, 15, 27** and **34** did not change dramatically (± 0.1 ppm) over a temperature range of 40°. This observation further suggested that the S-conformation is thermodynamically preferred in such compounds.

Ikehara [7b] was able to draw a firm conclusion that the conformation of the sugar moiety in nucleosides could be predicted by electronegativity (inductive effect) of the 2'-substituent, when he examined the ^{13}C nmr of these nucleosides. Thus, we calculated the electronegativity (E) of **5** from its ^{13}C nmr shift, utilizing the formula: E = [0.034]·[C-2'(ppm)] + 0.48. In Figure II, the results of **5** and other

Effects of Electronegativity on the ^{13}C nmr
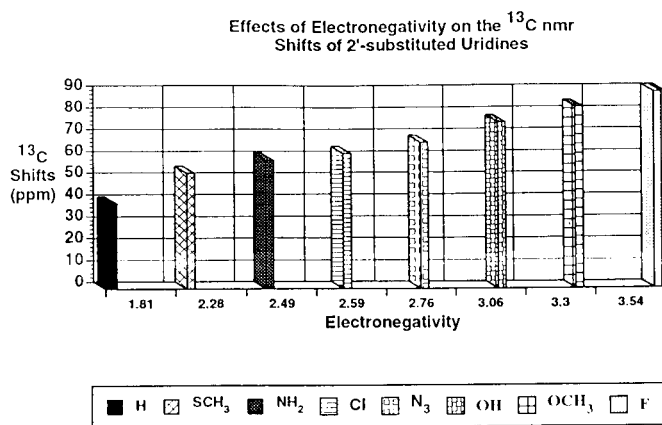Shifts of 2'-substituted Uridines

Figure II

2'-substituted nucleosides reported [29] in the literature are summarized. It seems reasonable that the sterically bulky group such as the 2'-methylthio group in **5**, would prefer a *quasi*-equatorial orientation, which results in an higher population of S-pucker. It is noteworthy, that the 2'-methylthio substituent moves the electronegativity of **5** closer to the 2'-deoxynucleoside (Figure II). Thus, it is safe to draw a conclusion that the influence of the 2'-methylthio substituent on the sugar conformation is mainly due to two factors: first, decreased electronegativity and sec-

ond, increased steric bulk (atomic radii). The restricted motions of the pentofuranosyl as seen here, may ultimately limit the flexibility of the oligonucleotide as a whole, thus giving an unique opportunity to modulate the properties of AO.

Conclusions.

We believe that the present synthesis constitutes a convenient approach for the conversion of ribonucleosides (U, 5-methyl-U, A and G) into the corresponding 2'-methylthio substituted derivatives. Indeed, it is reasonable to conclude that the described methodology is likely to prove to be of general value in the synthesis of various sugar modified nucleosides. To the best of our knowledge, this is a first example of highly S-puckered nucleoside analogs providing extra stiffness of the ribose pseudorotation. The rigid S-puckered oligonucleotides may play an important structural role in the designing of novel AO. The detailed study of incorporation of these nucleosides into various AO is in progress and will be a subject matter of another publication.

EXPERIMENTAL

Melting points (uncorrected) were determined in a Thomas-Hoover capillary melting-point apparatus. Elemental analyses were performed by Quantitative Technologies, Inc., Bound Brook, NJ. Thin layer chromatography (tlc) was performed on plates of silica gel 60F-254 (EM Reagents). Silica gel (ICN32/63; 60Å) was used for flash column chromatography. All solvents used were reagent grade. The detection of nucleoside components on tlc was by uv light, and with 10% sulfuric acid in methanol spray followed by heating. Evaporations were conducted under reduced pressure, with the water bath below 30°. Ultraviolet (uv) spectra were recorded with a HP 8452A spectrophotometer. Nuclear magnetic resonance spectra were collected on a Varian Unity 400 MHz spectrometer. Data sets for one-dimensional experiments were collected typically as 11000 complex points over a sweep-width of 3800 Hz with a recycle time of six seconds for a total of 32 transients, then zero-filled to 16k points and weighted with an exponential line broadening of 0.5 Hz before fourier transformation. Homonuclear two-dimensional spectra were collected as 1024 x 256 hypercomplex arrays, with recycle times of 0.64 s of TOCSY and 1.94 s for NOESY. Data were weighted with a shifted sine bell in each dimension before zero-filling to 1024 x 1024 points and fourier transformation. HMQC's were collected as 1024 x 128 hypercomplex arrays, with recycle times of 1.74 s. Data sets were weighted with a 111.4 ms gaussian function in the ^{1}H dimension and approximately 4.1 ms gaussian function in the ^{13}C dimension before fourier transformation. The chemical-shift values are expressed in parts per million (ppm) δ values, relative to tetramethylsilane as the internal standard. The signals in Tables are described as s (singlet), d (doublet), t (triplet), q (quartet) and m (multiplet). Preparative hplc was performed utilizing the waters 600E system.

Starting Materials.

The following compounds were prepared according to literature procedures: 5-methyluridine **2** [30]; 2,2'-anhydrouridine **3**

[15]; 9-[3,5-O-(1,1,3,3-tetraisopropydisiloxane-1,3-diyl)-2-O-trifluoromethanesulfonyl-β-D-arabino-furanosyl]adenine **23** [23]; 9-[3,4-O-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-2-O-trifluoromethanesulfonyl-β-D-arabino-furanosyl]guanine **32** [11].

Experimental Methods Related to Reaction Schemes I-II and Table I.

Method a.

2,2'-Anhydro-5-methyluridine (**4**).

A mixture of **2** (16.77 g, 69.2 mmoles) and diphenyl carbonate (17.8 g, 83.1 mmoles) in hexamethylphosphoramide (HMPA) (175 ml) was heated at 70° until a clear solution was obtained (~0.5 hour). The solution was cooled to room temperature and sodium bicarbonate (0.1 g, 2.1 mmoles) was added. The reaction mixture was further stirred at 150° for 2 hours, cooled to room temperature and poured into ice-water (150 ml). The solution was extracted with dichloromethane (2 x 250 ml) and the aqueous layer concentrated to furnish an oil. The residue was dissolved in ethanol (200 ml) and poured into vigorously stirred diethyl ether (1.5 ℓ) to precipitate a white powder. The precipitate was filtered, washed with diethyl ether (2 x 100 ml), and dried to furnish crude **4** (77%).

Method b.

2'-Deoxy-2'-methylthiouridine (**5**) and 2'-Deoxy-2'-methylthio-5-methyluridine (**6**).

Methanthiol gas (15.74 g, 327 mmoles) [31] was condensed into a stirred mixture of **3** (15.5 g, 68.2 mmoles) and N^1,N^1,N^3,N^3-tetramethylguanidine (42.7 ml, 341 mmoles) in dry dimethylformamide (150 ml) at −20°. The reaction mixture was heated in a pressure vessel at 60° for 12 hours, cooled to room temperature and concentrated to furnish a gummy residue. Silica gel chromatography (dichloromethane-methanol, 100:0 → 95:5) of the residue and crystallization from ethanol-diethyl ether gave **5** (14.11 g, 75%) as white powder.

An analogous procedure was utilized to convert **4** into **6**.

Method c.

3',5'-Di-O-acetyl-2'-deoxy-2'-methylthiouridine (**7**) and 3',5'-Di-O-acetyl-2'-deoxy-2'-methylthio-5-methyluridine (**8**).

Acetic anhydride (9.2 g, 75.5 mmoles) was added to a solution of **5** (9.0 g, 32.8 mmoles) and 4-dimethylaminopyridine (50 mg) in dry pyridine (50 ml) and the reactants were allowed to stir at room temperature for 12 hours. Pyridine was evaporated and the residue purified by flash chromatography (dichloromethane-methanol, 100:0 → 99:1) gave **7** (8.3 g, 76%). Crystallization of this material from ethanol gave **7** as colorless needles.

An analogous acetylation of **6** gave **8**.

Method d.

2'-Deoxy-2'-(methylsulfinyl)uridine (**9**), 2'-Deoxy-2'-(methylsulfinyl)-5-methyluridine (**10**) and 2'-Deoxy-2'-(methylsulfinyl)cytidine (**18**).

To a cooled (−15°) mixture of **5** (1 g, 3.65 mmoles) in ethanol (25 ml) was added 3-chloroperoxybenzoic acid (60%, 1.26 g, 3.65 mmoles). After 45 minutes of stirring at −15°, methanol (10 ml) was added at room temperature and the mixture was concentrated. The residue was purified by flash chromatography (dichloromethane-methanol, 9:1 → 6:4) to furnish **9** (0.5 g, 48%) as white powder.

In a similar manner **6** and **15** were oxidized to **10** and **18**, respectively.

Method e.

2'-Deoxy-2'-(methylsulfonyl)uridine (**11**) and 2'-Deoxy-2'-(methylsulfonyl)-5-methyluridine (**12**).

A mixture of **5** (1 g, 3.65 mmoles) and 3-chloroperoxybenzoic acid (60%, 5.04 g, 14.6 mmoles) in ethanol (30 ml) was stirred at ambient temperature. After 2 hours, a white precipitation occurred, which was filtered and washed with cold ethanol (2 x 5 ml) to furnish **11** (0.76 g, 68%).

In an analogous manner **6** was oxidized to **12**.

Method f.

1-[3',5'-Di-O-acetyl-2'-deoxy-2'-(methylthio)-β-D-ribofuranosyl]-4-(1,2,4-triazol-1-yl)pyrimidin-2-(1H)-one (**13**) and 1-[3',5'-Di-O-acetyl-2'-deoxy-2'-(methylthio)-β-D-ribofuranosyl]-4-(1,2,4-triazol-1-yl)pyrimidin-5-methyl-2-(1H)-one (**14**).

To a cold (0°) stirred solution of **8** (5.7 g, 16.5 mmoles) in dry pyridine (75 ml) was added phosphorus oxychloride (3.5 ml, 38.1 mmoles) over a period of 15 minutes. After 20 minutes, a solution of 1,2,4-triazole (8.0 g, 116 mmoles) in dry pyridine (25 ml) was added to the reaction mixture at 0°. The resulting solution was stirred at ambient temperature for 16 hours. The dark-red solution was added slowly, with vigorous stirring, to ice-water (1 ℓ), and the mixture obtained was extracted with dichloromethane (2 x 250 ml). The extract was washed successively with saturated aqueous sodium hydrogen carbonate (3 x 250 ml) and water (2 x 250 ml), dried (magnesium sulfate), and concentrated. The residue was purified by silica gel chromatography (ethyl acetate-hexanes, 3:2) to furnish **14** (4.89 g, 70%). The product was crystalized from ethanol as fine needles.

In a similar way **7** was triazolated to furnish **13**.

Method g.

2'-Deoxy-2'-methylthiocytidine (**15**) and 2'-Deoxy-2'-methylthio-5-methylcytidine (**16**).

A mixture of **13** (5.16 g, 12.5 mmoles) and aqueous ammonium hydroxide-1,4-dioxane (30%, 1:1, 60 ml) was stirred at room temperature for 12 hours. The reaction mixture was concentrated and the residue purified by flash chromatography (dichloromethane-methanol, 9:1 → 85:15) to furnish **15** (2.92 g, 86%).

Following this method, nucleoside **14** was deblocked to furnish **16**.

Method h.

2'-Deoxy-2'-methylthio-O^4-methyluridine (**17**).

A mixture of **13** (4.8 g, 11.7 mmoles) and saturated (0°) methanolic ammonia (50 ml) was stirred in a pressure bottle at room temperature for 16 hours. The reaction mixture was concentrated and the residue purified by chromatography (dichloromethane-methanol, 100:0 → 9:1) furnished a nonpolar product **17** (0.74 g, 23%) and polar nucleoside **15** (0.3 g, 9%).

Method i.

9-[3-deoxy-3-(methylthio)-β-D-xylofuranosyl]adenine (**25**).

To a stirred solution of **22** (4 g, 6.8 mmoles) in dry DMF (75 ml) was added sodiumthiomethoxide (0.62 g, 8.85 mmoles) at room temperature. After 12 hours, the solution was concentrated and the residue purified by silica gel chromatography (dichlorometh-

ane-methanol, 99:1 → 9:1) to furnish **25** (1.54 g, 76%) as crystalline (ethanol) product.

Method j.

9-[2-Deoxy-2-(methylthio)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]adenine (**26**) and 9-[2-Deoxy-2-(methylthio)-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)-β-D-ribofuranosyl]guanine (**33**).

Methanethiol gas (0.30 g, 6.25 mmoles) [31] was condensed in a mixture of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (1.25 ml, 8.36 mmoles) in dry DMF (25 ml) at −25°. The foregoing solution was then added dropwise to a stirred solution of **23** (1.34 g, 2.1 mmoles) in dry DMF (25 ml) at −30°. After 15 minutes the reaction mixture was allowed to warm-up to −5° and poured into ice-water (50 ml). The aqueous solution was extracted with dichloromethane (3 x 50 ml) and the combined organic layer was dried (magnesium sulfate). The solution was concentrated and the residue purified by chromatography (hexanes-ethyl acetate, 7:3 → 1:1) to furnish **26** (1.05 g, 95%).

In an analogous way **32** was transformed to **33**.

Method k.

9-[2-Deoxy-*erythro*-pent-1-eno-3,5-*O*-(1,1,3,3-tetraisopropyldisiloxane-1,3-diyl)furanosyl]adenine (**28**).

Methanethiol gas (3.0 g) [31] was condensed into dry dichloromethane (100 ml) at −25° to prepare a stock solution. DBU (0.46 ml, 3.12 mmoles) was added to the methanethiol stock solution (1.4 ml) at 0° and allowed to warm-up to room temperature. A solution of **23** (0.5 g, 0.78 mmole) in dry dichloromethane (5 ml) was added to the mixture of DBU/methanethiol at room temperature and stirred for 1 hour. The reaction mixture was poured into ice-water (50 ml) and the solution extracted with dichloromethane (3 x 25 ml). Combined organic layers were dried (magnesium sulfate) and concentrated. The residue was purified by chromatography (hexanes-ethyl acetate, 7:3 → 4:6) to provide **28** (0.38 g, 99%).

Method l.

2′-Deoxy-2′-methylthioadenosine (**27**) and 9-[2-Deoxy-*erythro*-pent-1-enofuranosyl]adenine (**29**).

A solution of 1 *M* tetrabutylammonium fluoride in tetrahydrofuran (6.11 ml) was added to a stirred solution of **26** (1.1 g, 2.0 mmoles) in dichloromethane (20 ml) at room temperature. After 30 minutes, the mixture was evaporated and the residue purified by chromatography (dichloromethane:methanol, 100:0 → 99:1) to furnish **27** (0.44 g, 73%), which crystallized from ethanol.

In a similar manner, nucleoside **28** was deblocked to **29**.

Method m.

2′-Deoxy-2′-methylthioguanosine (**34**).

A solution of hydrogen fluoride-pyridine (Aldrich, 0.5 ml) was added to a stirred solution of **33** (0.2 g, 0.36 mmole) in tetrahydrofuran:pyridine (20 ml:5 ml) at room temperature. After 2 hours, the mixture was evaporated and the residue crystallized from dichloromethane:methanol to furnish **34** (0.038 g, 33%).

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