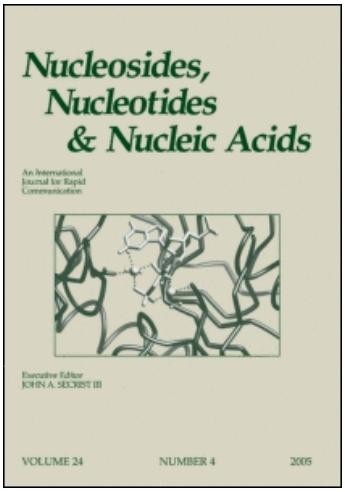

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Aminolysis of 2'-Deoxyinosine Aryl Ethers: Nucleoside Model Studies for the Synthesis of Functionally Tethered Oligonucleotides

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AMINOLYSIS OF 2'-DEOXYINOSINE ARYL ETHERS: NUCLEOSIDE MODEL STUDIES FOR THE SYNTHESIS OF FUNCTIONALLY TETHERED OLIGONUCLEOTIDES

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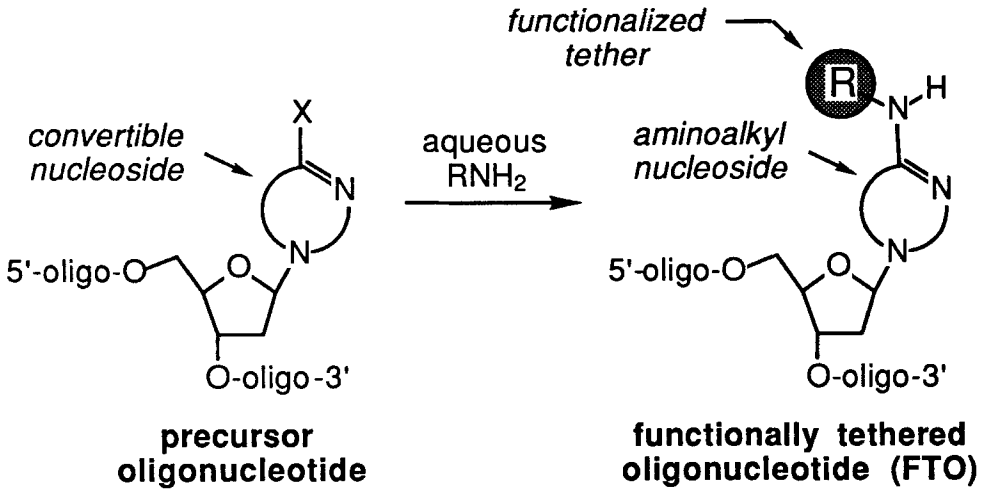
Abstract: Several O^6-aryl-2'-deoxyinosines were synthesized and found to undergo conversion to N^6-substituted-2'-deoxyadenosines upon treatment with aqueous amines. The kinetics for reaction of these nucleosides with various amines suggests that O^6-phenyl- and O^6-(*p*-nitrophenyl)-2'-deoxyinosine are suitable "convertible nucleoside" precursors for the site-specific introduction of functionally tethered 2'-deoxyadenosines into DNA.

Oligonucleotides bind to complementary nucleic acid sequences with a high degree of specificity, but lack functionality to report binding to an outside observer. To overcome this limitation, synthetic approaches have been devised to tether reporter groups to oligonucleotides site-specifically during automated DNA synthesis.[1] Most strategies involve attachment of a nucleophile-bearing tether to a monomeric nucleoside, which is incorporated into the DNA during automated synthesis, and subsequent linkage of the reporter to the tethered nucleophile.[2] Our laboratory has developed an alternative, convergent strategy for the synthesis of functionally tethered oligonucleotides (FTOs), which allows the functionalized tether to be attached at the *end* of the DNA synthesis.[3] In this approach, a nucleoside capable of undergoing selective aminolytic conversion to a modified base ("convertible nucleoside") is incorporated site-specifically into an oligonucleotide, and the functionalized tether is

SCHEME I

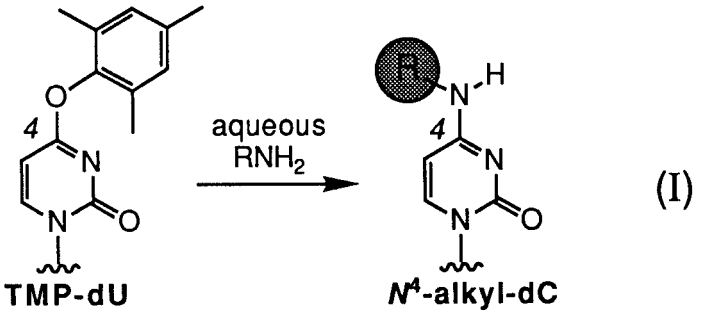


attached by treatment of the completed sequence with an aqueous amine (Scheme I). This route enables one to synthesize a wide variety of FTOs by reacting a single precursor oligonucleotide with different amines, and eliminates the need to carry out a separate synthesis for each tethered monomer.

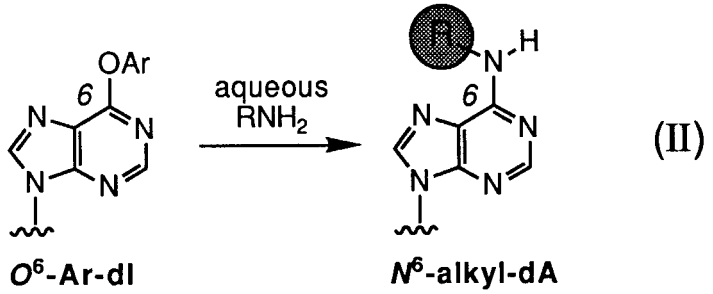
We have used the convertible nucleoside O^4-(2,4,6-trimethylphenyl)-2'-deoxyuridine (**TMP-dU**, eq I) to synthesize FTOs with tethers at the exocyclic amine group (N^4) of 2'-deoxycytidine residues.[3] We wished to extend this chemistry to the attachment of tethers at the exocyclic amine of 2'-deoxyadenosine (dA). A dA-convertible nucleoside having the following properties is required: (i) stability to the conditions of automated DNA synthesis, and (ii) ability to undergo clean reaction with amines under conditions tolerated by DNA. Because an aryl ether fulfilled these critieria in the dU/dC series, we first synthesized O^6-aryl-2'-deoxyinosines (O^6-Ar-dI) to evaluate their reactivities (eq II).[4] These nucleoside model studies have allowed us to select derivatives suitable for the synthesis of oligonucleotides functionally-tethered at dA.

Results.

Nucleoside synthesis. O^6-aryl-2'-deoxyinosines (**4a-c**) were prepared from 2'-deoxyinosine (**1**) via the known O^6-enol sulfonate ester **2**

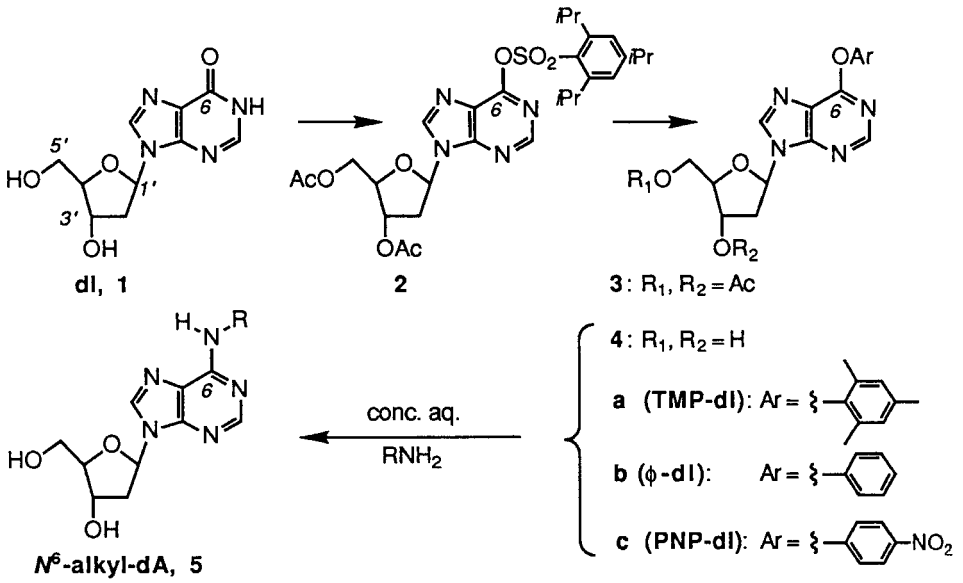


(I)



(II)

SCHEME II



(Scheme II).[5] Trimethylamine-mediated substitution of the enol sulfonate by phenols proceeded smoothly in 75 to 97% yield to afford the aryl enol ethers **3a-c**, which were then deacetylated to provide **4a-c**. Care was taken to avoid causing either exchange of the aryl ether to a methyl ether during deacetylation with methoxide or cleavage of the glycosidic bond under the acidic conditions of the subsequent workup.

Aminolysis of convertible nucleosides. The following considerations were used to evaluate nucleosides **4** for use in FTO synthesis: (1) aminolysis of DNA should be essentially complete in < 24 h to minimize decomposition of the oligonucleotide (by thermal depurination or base-catalyzed phosphodiester hydrolysis). For the reaction to be > 98% complete, it must proceed at least 6 half-lives, so a maximum half-life of 4 hours is required. The reaction should proceed cleanly, so as to avoid the need for purification of closely related oligonucleotides after attachment of the tether. To assess the ability of convertible nucleosides **4a-c** to serve as precursors to tethered dA's, the nucleosides were treated with aqueous amines under conditions similar to those used for the synthesis of dC-tethered FTO's. During the reaction, aliquots were removed for HPLC analysis of the extent of conversion (Table 1) and the product distribution (see below). In our experience, the aminolysis half-lives of monomeric nucleosides **TMP-dU** and **4** are somewhat shorter than the half-lives of the same nucleosides in DNA,[6] so the values listed in Table 1 may be considered a lower limit.

The reaction kinetics of convertible nucleosides **4a-c** with a variety of amines are compiled in Table 1. All reactions obeyed pseudo-first-order kinetics (data not shown), due to the large excesses of amine used. Aminolysis rates varied widely, depending on the nature of the aryl ether substituent and the nucleophilicity of the amine. O^6-(trimethylphenyl)-2'-deoxyinosine (**TMP-dI**, **4a**) was surprisingly stable: it underwent only sluggish aminolysis with relatively nucleophilic amines (e.g., methylamine), and essentially no reaction with less nucleophilic ones (e.g., glycine). In contrast, the reaction of the trimethylphenyl ether of dU (**TMP-dU**) with amines is rapid, occurring approximately 200 times faster than the reaction of **TMP-dI** under similar conditions.[6] The extended reaction times for aminolytic conversion of **TMP-dI** make this compound unsuitable for use in the synthesis of FTOs.

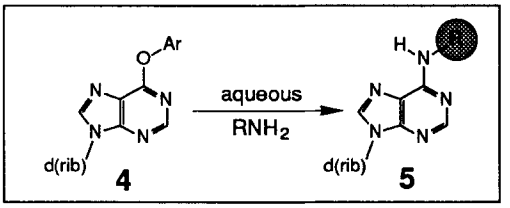
O^6-(phenyl)-2'-deoxyinosine (**ϕ-dI, 4b**) underwent reaction with a wide variety of amines, and had reaction kinetics quite similar to those of **TMP-dU**. The reaction of **ϕ-dI** with non-nucleophilic amines is slow (for example, the reaction with glycine proceeds with a half-life of 10 h; see Table 1), although the sluggishness of these reactions may be attributed in part to the poor solubility of the amine in water. Nonetheless, these data indicate that O^6-phenyl-dI (**4b**) is sufficiently reactive to be used for FTO synthesis, particularly when nucleophilic amines are used for the tether.

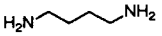
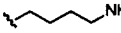
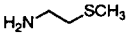
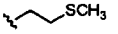
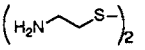
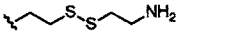
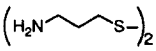
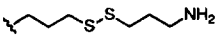
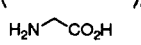
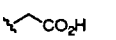
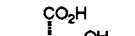
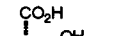
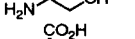
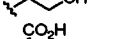
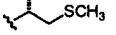
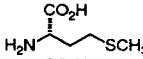
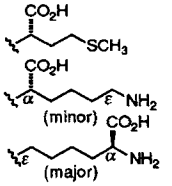
O^6-(*p*-nitrophenyl)-2'-deoxyinosine (**PNP-dI, 4c**) was the most reactive of the three convertible nucleosides examined in this study, undergoing aminolysis at rates ~6-25 times faster than **ϕ-dI** (Table 1). Even amines that show poor reactivity with **TMP-dI** or **ϕ-dI** displace the *p*-nitrophenyl ether moiety of **PNP-dI** with half-reaction times of < 1 h (the half-time for reaction with serine is 30 min). **PNP-dI** was therefore sufficiently reactive to be used as a convertible nucleoside in FTO synthesis.

Product distribution. In almost all cases, aminolysis of **4** cleanly gave rise to a single product, **5**. Because the reaction is carried out under basic conditions, depurination of the products is not a problem (purines are prone to acid-catalyzed glycosidic bond cleavage). Competitive displacement of the aryl ether by hydroxide ion (evidenced by the formation of dI) is the main side reaction of concern. But deoxyinosine was observed by HPLC analysis in only two cases, even at amine concentrations as low as 0.25 M: 5% dI was formed during the reaction of ammonium hydroxide with **ϕ-dI**, and 10% hydrolysis occurred during treatment of **TMP-dI** with 1 M diaminobutane (Table 1). Other potential side reactions include the formation of dimeric nucleosides from reactions with diamines, or ethers by reaction with the -OH of serine. At the concentrations used here, products of such reactions were not observed (although much lower concentrations of diamines can be used to favor dimer formation). Reaction with lysine occurs at both amine groups, with the major product resulting from addition of the more reactive ε-amine (Table 1).

Stability of convertible nucleosides to the conditions of automated DNA synthesis. During automated DNA synthesis (phosphoramidite method), the growing oligonucleotide chain is exposed to conditions that could cause side-reactions of convertible nucleosides. Of particular

TABLE 1. Conversion of O^6-aryl-2'-deoxyinosines (**4a-c**) to N^6-alkyl-2'-deoxyadenosines with aqueous amines at 65 °C.



H_2NR	conc. (*M*)	half-life (hr) TMP-dI (4a)	ϕ-dI (4b)	PNP-dI (4c)	R
H_3N	14.0	~120	2[c]	n.d.	
H_2NCH_3	7.0	~ 8	0.1	n.d.	
	1.0	80[d]	0.2	n.d.	
	1.0	n.r.	2.25	<1	
	0.25 [a,b]	n.d.	2	n.d.	
	0.25 [a,b]	n.d.	1	n.d.	
	1.0 [a]	n.r.	2.0	0.3	
	1.0 [a]	n.d.	10	0.5	
	1.0 [a]	n.d.	21	0.8	
	0.7 [a]	n.d.	10.5	0.5	
	1.0 [a]	n.d.	2[e]	0.3[e]	

[a] The reaction mixture contained 10% triethylamine to ensure that the amine was present as the free base.

[b] $(H_2NCH_2CH_2S\text{-})_2\cdot 2HCl$ and $(H_2NCH_2CH_2CH_2S\text{-})_2\cdot 2HBr$ were used.

[c] Formation of 5% dI was observed.

[d] Formation of 10% dI was observed.

[e] The ratios of minor to major product were 20:80 with **4b** and 40:60 with **4c**.

n.r.: No reaction was observed after incubation for 19 hours.

n.d.: Half-lives were not determined.

concern are the reagents for oxidation (I_2/lutidine/H_2O), phosphoramidite activation (tetrazole/CH_3CN), and detritylation (3% CH_3CO_2H/CH_3CN). Nucleosides **4b** and **4c** were exposed to these conditions for a length of time corresponding to approximately 60 synthesis cycles (30 min) in order to examine their stabilities.[7] After this, the reactions were quenched and the product isolated for examination by ^1H NMR. In all cases, the starting nucleosides were recovered unchanged. The stability of the *p*-nitrophenyl ether **4c** to the acidic detritylation solution was unexpected, since purine 2'-deoxyribosides are known to undergo acid-catalyzed glycosidic bond cleavage. The results of these tests indicated that all of the nucleosides **4** should be stable during automated oligonucleotide synthesis.

Discussion. The objective of this study was to obtain a convertible nucleoside that would undergo clean and rapid reaction with alkylamines to furnish N^6-alkyl-dA derivatives and would remain intact during automated DNA synthesis. Two such molecules were identified on the basis of these results: O^6-phenyl-2'-deoxyinosine (**4b**) and O^6-(4-nitrophenyl)-2'-deoxyinosine (**4c**); a third nucleoside, namely O^6-(2,4,6-trimethylphenyl)-2'-deoxyinosine (**4a**) failed to undergo sufficiently rapid aminolytic conversion to be used in FTO synthesis.

The aminolytic conversion of dI aryl ethers can be used to generate a diverse array of functionalized 2'-deoxyadenosines. As shown in Table 1, tethers containing amine, alcohol, thioether, thiol (protected as the mixed disulfide), and carboxyl groups, are readily appended to the exocyclic amino function of dA. This method should be useful for synthesizing nucleoside derivatives that possess interesting biological activity, such as those effective in antiviral and anticancer chemotherapy.[8] Because the aminolysis reaction takes place in aqueous solution, synthesis of highly functionalized nucleoside derivatives (nucleopeptides, for example) may be accomplished without complicated protection/deprotection schemes. While the high concentrations of amines used in this study (0.25-7 *M*) might preclude the use of very expensive amines, it is likely that the *p*-nitrophenyl ether **4c** will undergo substitution at significantly lower amine concentrations.

The aminolysis rates and stability studies reported above strongly suggest that convertible nucleosides **4b** and **4c** will serve as effective vehicles for the site-specific incorporation of functionalized tethers into

DNA. In studies reported elsewhere, we have synthesized oligonucleotides containing ϕ-**dI** and shown that they undergo clean and efficient conversion to the corresponding FTOs upon treatment with diamines.[9] A further exciting possibility is that the differences in reactivity between **4b** and **4c** may be exploited to generate FTOs bearing two chemically distinct tethers.

Experimental Procedures

General Methods: ^1H NMR spectra were recorded on a Bruker AM-300, AM-400, or AM-500 spectrometer and are reported in parts per million (ppm) with reference to the solvent, $CHCl_3$ (δ 7.24 ppm) or DHO (δ 4.76 ppm). ^{13}C NMR spectra were recorded on a Bruker AM-400 (100 MHz) or AM-500 (125 MHz) spectrometer and are reported in ppm with reference to $CDCl_3$ (δ 77.0 ppm). High-resolution mass spectra (FAB on a glycerol matrix) were obtained by Dr. Andrew Tyler of the Harvard University Chemistry Department Mass Spectrometry Facility on a Kratos MS50 mass spectrometer. IR spectra were recorded on a Nicolet 5PC FT-IR spectrometer. HPLC was performed on a Hewlett-Packard 1090 liquid chromatograph equipped with a diode array detector. UV spectra of compounds are automatically obtained as they elute from the column during each run. Gradient A (analytical runs): a 4.6 x 250 mm Beckman C_{18} Ultrasphere column was used with a 10 minute gradient from 0.02 M monobasic potassium phosphate, pH 5.4, to 75:25 methanol:water, followed by MeOH:H_2O (75:25) for 5 minutes at a flow rate of 2 ml/min. Gradient B (preparative runs): a 10 x 250 mm Beckman C_{18} Ultrasphere column was used with a flow rate of 4 ml/min during a 15 minute gradient from 0.02 M KH_2PO_4, pH 5.4, to 75:25 MeOH:H_2O, then 5 minutes in 75:25 MeOH:H_2O.

3',5'-*O*-diacetyl-*O*^{6}-(triisopropylbenzenesulfonyl)-2'-deoxyinosine (2). 3',5'-*O*-diacetyl-2'-deoxyinosine (650 mg, 1.93 mmol. Seela et al, *Helv. Chim. Acta* **1987**, *70*, 1649.) was dissolved in 25 ml of methylene chloride. Triispropylbenzenesulfonyl chloride (1.75 g, 5.8 mmol, 3 equivalents), 4-dimethylaminopyridine (18 mg, 0.15 mmol) and triethylamine (1.95 ml, 14 mmol) were added. The solution was stirred for 2 hours under nitrogen at room temperature. The solvent was

removed *in vacuo* and the two products (formed in a 1:1 ratio) were separated by flash chromatography in 50:50 ethyl acetate: hexanes. (R_f(**2**) = 0.40 in 9:1 methylene chloride: methanol.) This yielded 362 mg (0.60 mmol, 31%) of **2** as a white solid. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.21-1.26 ppm (m, 18 H, C*H*$_3$'s), 2.05 (s, 3 H, C(O)-C*H*$_3$), 2.11 (s, 3 H, C(O)-C*H*$_3$), 2.64 [ddd (~12, 6.0, 2.6 Hz), 1 H, H2'), 2.85-2.99 (m, 2 H, H2' and *p*-C*H*$(CH_3)_2$), 4.27-4.40 (m, 5 H, H4', H5', and *o*-C*H*$(CH_3)_2$), 5.41 [br d (5.6 Hz), 1 H, H3'], 6.45 [dd (6.9 Hz), 1 H, H1'], 7.20 (s, 2 H, aryl H's), 8.21 (s, 1 H, H8), 8.56 (s, 1 H, H2).

3',5'-*O*-diacetyl-*O*^{6}-phenyl-2'-deoxyinosine (3b). 3',5'-*O*-diacetyl-*O*^{6}-(triisopropylbenzenesulfonyl)-2'-deoxyinosine (**2**, 500 mg, 0.828 mmol) was dissolved in 10 ml methylene chloride. Phenol (480 mg, 5.11 mmol, 6.2 equiv) was added, the solution was cooled to 0 °C, and trimethylamine was bubbled through it for 10 minutes. After addition of triethylamine (0.400 ml, 2.9 mmol) and stirring for 2 hours at 0 °C, the solvent was removed *in vacuo*. Purification of the product by flash chromatography with 60:40 ethyl acetate: hexanes followed by ethyl acetate (R_f = 0.15 in 50:50 ethyl acetate: hexanes) yielded 330 mg (0.801 mmol, 97%) of 3',5'-*O*-diacetyl-*O*^{6}-phenyl-2'-deoxyinosine. ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.05 ppm (s, 3H, C(O)-C*H*$_3$), 2.09 (s, 3 H, C(O)-C*H*$_3$), 2.62 [ddd (14.2, 6.0, 2.6 Hz), 1 H, H2'], 2.94-3.06 (m, 1 H, H2'), 4.30-4.41 (m, 3 H, H4' and H5'), 5.43 [br d (6.3 Hz), 1 H, H3'], 6.46 [dd (7.7, 6.2 Hz), 1 H, H1'], 7.20-7.45 (m, 5 H, aryl H's), 8.19 (s, 1 H, H8), 8.46(s, 1 H, H2).

3',5'-*O*-diacetyl-*O*^{6}-(trimethylphenyl)-2'-deoxyinosine (3a) and **3',5'-*O*-diacetyl-*O*^{6}-(*para*-nitrophenyl)-2'-deoxyinosine (3c)** were prepared by reaction of **2** with trimethylphenol or *p*-nitrophenol using the same method as for synthesis of **3b.** Yields were 75% and 94% respectively. R_f (**3a**) = 0.14 and R_f (**3b**) = 0.28 in 60:40 ethyl acetate: hexanes. **3a** ^{1}H NMR ($CDCl_3$, 250 MHz): δ 2.07 ppm (s, 9 H, *o*-C*H*$_3$'s and C(O)-C*H*$_3$), 2.11 (s, 3 H, C(O)-C*H*$_3$), 2.27 (s, 3 H, *p*-C*H*$_3$), 2.65 [ddd (14.1, 6.0, 2.7 Hz), 1 H, H2'], 2.96-3.08 (m, 1 H, H2'), 4.31-4.43 (m, 3 H, H4' and H5'), 5.42-5.46 (m, 1 H, H3'), 6.49 [dd (7.7, 6.0 Hz), 1 H, H1'], 6.91 (s, 2 H, aryl H's), 8.18 (s, 1 H, H8), 8.43 (s, 1 H, H2). **3c** ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.08 ppm (s, 3 H, C(O)-C*H*$_3$), 2.13 (s, 3 H, C(O)-C*H*$_3$), 2.67 [ddd (14.1, 6.0, 2.7m Hz), 1 H, H2'), 2.96-3.06 (m, 1 H,

H2'), 4.32-4.43 (m, 3 H, H4' and H5'), 5.45 [br d (6.3 Hz), 1 H, H3'], 6.49 [dd (7.6, 6.1 Hz), 1 H, H1'), 7.44 [d (9.0 Hz) , 2 H, *o*-aryl H's), 8.24 (s, 1 H, H8), 8.33 [d (9.0 Hz, 2 H, *m*-aryl H's], 8.51 (s, 1 H, H2).

O^6-phenyl-2'-deoxyinosine (4b). 3',5'-*O*-diacetyl-O^6-phenyl-2'-deoxyinosine (370 mg, 0.90 mmol) was dissolved in 2 ml methanol. Potassium carbonate (50 mg) was added and the reaction mixture was stirred for 5 minutes at room temperature (until only product could be detected by TLC in 6:1 methylene chloride: methanol, R_f (**4b**) = 0.35). The potasssium carbonate was filtered off and the resulting solution was neutralized by stirring over Dowex 50W proton resin for 10 minutes. The resin was filtered off and the filtrate concentrated *in vacuo*. The product was purified by flash chromatography with 9:1 methylene chloride: methanol to yield 278 mg (0.85 mmol, 95%) of O^6-phenyl-2'-deoxyinosine. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.37 ppm [dd (13.4, 5.4 Hz), 1 H, H2'], 2.98-3.03 (m, 2 H, H2' and 3'-O*H*), 3.77 [br dd (12, 12 Hz), 1 H, H5'], 3.95 [br d (13 Hz), 1 H, H5'], 4.23 (br s, 1 H, H4'), 4.78 [br d (4.6 Hz), 1 H, H3'], 5.90 [br d (12 Hz), 1 H, 5'-O*H*], 6.40 [dd (9.2, 5.5 Hz), 1 H, H1'), 7.22-7.47 (m, 5 H, aryl H's), 8.08 (s, 1 H, H8), 8.45 (s, 1 H, H2). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 40.8 ppm (C2'), 63.0 (C5'), 72.6 (C3'), 87.2 (C1'), 89.2 (C2'), 121.7 (*o*-C's), 123.0 (C5), 126.0 (*p*-C), 129.7 (*m*-C's), 142.8 (C8), 151.7 (C2), 152.0 (C4), 160.5 (C6). IR (neat): 3328 (br), 2928, 1609, 1453, 1225 cm^{-1}. λ_{max} = 251 nm. HRMS calcd for $C_{16}H_{16}O_4N_4$ (M + H) 329.1250, found 329.1271.

O^6-(trimethylphenyl)-2'-deoxyinosine (4a) and **O^6-(p-nitrophenyl)-2'-deoxyinosine (4c)** were prepared from **3a** and **3c** in 89% and 72% yield respectively, under the same reaction conditions as used for synthesis of **4b**. R_f (**4a**) = 0.26 and R_f (**4c**) = 0.51 in 9:1 methylene chloride: methanol. **4a** ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.07 ppm (s, 6 H, *o*-C*H*$_3$'s), 2.29 (s, 3 H, *p*-C*H*$_3$), 2.38 [dd (13.3, 5.5 Hz), 1 H, H2'], 3.02-3.08 (m, 1 H, H2'), 3.20 (br s, 1 H, 3'-O*H*), 3.77 [br dd (12, 12 Hz), 1 H, H5'], 3.95 [br d (12.7 Hz), 1 H, H5'], 4.24 (s, 1 H, H4'), 4.79 (br s, 1 H, H3'), 6.02 [br d (11 Hz), 1 H, 5'-O*H*], 6.40 [dd (9.3, 5.5 Hz), 1 H, H1'], 6.93 (s, 2 H, aryl H's), 8.07 (s, 1 H, H8), 8.42 (s, 1 H, H2). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 16.3 ppm (*o*-C's), 20.8 (*p*-C), 40.9 (C2'), 63.2 (C5'), 72.9 (C3'), 87.6 (C1'), 89.4 (C4'), 122.7 (C5), 129.4, 130.0, 135.7,

142.5 (C8), 146.9, 151.4 (C2), 152.0 (C4), 160.2 (C6). IR (neat): 3324 (br), 2923, 1603, 1578, 1456, 1223 cm^{-1}. λ_{max} = 253 nm. HRMS calcd for $C_{19}H_{25}O_4N_4$ (M + H) 371.1719, found 371.1691. **4c** ^1H NMR ($CDCl_3$, 300 MHz): δ 2.38 ppm [dd (13.4, 5.5 Hz), 1 H, H2'], 3.03-3.13 (m, 1 H, H2'), 3.81 [dd (11, 9.5 Hz), 1 H, H5'], 3.97 [br d (13 Hz), 1 H, H5'], 4.24 (s, 1 H, H4'), 4.82 (m, 1 H, H3'), 5.58 [dd (11, 2.2 Hz), 1 H, 5'-O*H*], 6.42 [dd (9.5, 5.4 Hz), 1 H, H1'], 7.44 [d (9.1 Hz), 2 H, *o*-aryl H's], 8.13 (s, 1 H, H8), 8.34 [d (9.1 Hz), 2 H, *m*-aryl H's), 8.49 (s, 1 H, H2). IR (neat): 3306 (br), 3117, 2926, 1570, 1522,1456, 1345, 1223 cm^{-1}. λ_{max} = 261 nm.

***N*^{6}-substituted-2'-deoxyadenosines (5)** were prepared in a typical procedure by treating 5 mg of an *O*^{6}-aryl-2'-deoxyinosine (**4a-c**) with 1 ml of an aqueous amine solution (see Table I for concentrations) at 65 °C in an Eppendorf tube. For amines that are available as salts (e.g. cystamine dihydrochloride) or that exist in the zwitterionic form (amino acids) 10% triethylamine was added to the reaction mixture to insure that the free base form would be present. Reaction progress was monitored by HPLC (Gradient A): at each time point, a 25 µl aliquot of the reaction mixture was removed and diluted to 1 ml with 0.02 M KH_2PO_4 buffer to quench the reaction. (If analysis could not be performed immeditately, samples were stored at -20 °C. Once anaylsis showed the reaction to be complete, the products were purified by HPLC (Gradient B).

***N*^{6}-methyl-2'-deoxyadenosine (5a).** ^1H NMR ($CDCl_3$, 300 MHz): δ 2.3 ppm (m, 1 H, H2'), 3.1 (m, 1 H, H2'), 3.2 (br s, 3 H, C*H*$_3$), 3.75-4.0 (m, 2 H, H5'), 4.2 (s, 1 H, H4'), 4.8 (d, 1 H, H3'), 6.3 (dd, 1 H, H1'), 7.8 (s, 1 H, H8), 8.35 (s, 1 H, H2). HRMS calcd for $C_{11}H_{15}O_3N_5$ (M + H) 266.1254, found 266.1270. λ_{max} = 263 nm.

***N*^{6}-(4-aminobutyl)-2'-deoxyadenosine (5b).** ^1H NMR (D_2O, 300 MHz): δ 1.61 ppm (br s, 4H, HN-$CH_2C\mathit{H}_2C\mathit{H}_2CH_2NH_2$), 2.35-2.42 (m, 1 H, H2'), 2.62-2.70 (m, 1 H, H2'), 2.88 (br s, 2H, C*H*$_2$NH$_2$), 3.44 (br s, 2 H, HN-C*H*$_2$), 3.60-3.68 (m, 2 H, H5'), 4.02 (br s, 1 H, H4'), 4.47 (br s, 1 H, H3'), 6.28 [dd (7.3, 6.4 Hz), 1 H, H1'], 8.03 (s, 1 H, H8), 8.09 (s, 1 H, H2). HRMS calcd for $C_{14}H_{22}O_3N_6$ (M + H) 323.1832, found 323.1844. λ_{max} = 265 nm.

***N*^{6}-(2-(methylthio)ethyl)-2'-deoxyadenosine (5c).** ^1H NMR (D_2O, 300 MHz): δ 1.98 ppm (s, 3 H, C*H*$_3$), 2.35-2.45 (m, 1 H, H2'), 2.63-2.73 (m, 1 H, H2'), 2.70 [tr (6.6 Hz), 2 H, C*H*$_2$S], 3.58-3.71 (m, 4 H, H5'

and HN–CH_2), 3.99-4.07 (m, 1 H, H4'), 4.47-4.51 (m, 1 H, H3'), 6.31 [dd (6.3, 7.0 Hz), 1 H, H1'], 8.07 (s, 1 H, H8), 8.11 (s, 1 H, H2). HRMS calcd for $C_{13}H_{19}O_3N_5S$ (M + H) 326.1289, found 326.1297. IR (neat): 3318 (br), 2919, 1620, 1294, 1231 cm^{-1}. λ_{max} = 265 nm.

***N*^{6}-(6-amino-3,4-dithiahexyl)2'-deoxyadenosine (5d).** ^{1}H NMR (D_2O, 300 MHz): δ 2.44-2.52 ppm (m, 1 H, H2'), 2.73-2.80 (m, 1 H, H2'), 2.88 [t (5.9 Hz), 2 H, SCH_2], 3.01 [t (6.1 Hz), 2 H, SCH_2], 3.25 [t (6.3 Hz), 2 H, CH_2NH_2], 3.65-3.78 (m, 2 H, H5'), 3.85 (br s, 2 H, HN-CH_2), 4.11 (br s, 1 H, H4'), 4.57 (br s, 1 H, H3'), 6.39 [dd (7.0, 7.0 Hz), 1 H, H1'], 8.17 (s, 1 H, H8), 8.21 (s, 1 H, H2). HRMS calcd for $C_{14}H_{22}O_3N_6S_2$ (M + Na) 409.1093, found 409.1101. λ_{max} = 267 nm.

***N*^{6}-(8-amino-4,5-dithiaoctyl)2'-deoxyadenosine (5e).** ^{1}H NMR (D_2O, 300 MHz): δ 1.94 ppm [t (7.4 Hz), 2 H, $SCH_2CH_2CH_2NH_2$], 2.03 [t (6.9 Hz), 2H, $SCH_2CH_2CH_2NH_2$], 2.48 (m, 1H, H2'), 2.66 [t (7.0 Hz), 2H, $SCH_2CH_2CH_2NH_2$], 2.79 [t (7.0 Hz), 2H, $SCH_2CH_2CH_2NH_2$], 2.98 [t (7.6 Hz), 2H, $SCH_2CH_2CH_2NH_2$], 3.63 (br s, 2H, -NHCH_2CH_2S), 3.68-3.78 (m, 2H, H5'), 4.11 (br s, 1H, H4'), 4.57 (br s, 1H, H3'), 6.40 [dd (6.8, 7.3 Hz), 1H, H1'], 8.16 (s, 1H, H8), 8.20 (s, 1H, H2). HRMS calcd for $C_{16}H_{26}O_3N_6S_2$ (M + H) 415.1586, found 415.1592. λ_{max} = 265 nm.

***N*^{6}-carboxylmethyl-2'-deoxyadenosine (5f).** ^{1}H NMR (D_2O, 300 MHz): δ 2.35-2.45 ppm (m, 1H, H2'), 2.64-2.72 (m, 1H, H2'), 3.58-3.72 (m, 2H, H5'), 3.98 and 4.03 (2 br s, 3H, α-CH_2 and H4'), 4.46-4.53 (m, 1H, H3'), 6.32 [dd (8.4, 8.4 Hz), 1H, H1'], 8.07 (s, 1H, H8), 8.13 (s, 1H, H2). HRMS calcd for $C_{11}H_{15}O_3N_5$ (M + K) 348.0710, found 348.0722. IR (neat): 3235 (br), 2917, 2849, 1576 cm^{-1}. λ_{max} = 265 nm.

***N*^{6}-(1-(S)-carboxy-2-hydroxyethyl)2'-deoxyadenosine (5g).** ^{1}H NMR (D_2O, 300 MHz): δ 2.34-2.47 ppm (m, 1H, H2'), 2.62-2.74 (m, 1H, H2'), 3.58-3.68 (m, 2H, H5'), 3.89 (br s, 2H, CH_2OH), 4.02 (br s, 1H, H4'), 4.49 (br s, 1H, H3'), ~4.75 (under DHO peak, α-H of amino acid), 6.32 [dd (8.2, 8.3 Hz), 1H, H1'], 8.08 (s, 1H, H8), 8.10 (br s, 1H, H2). HRMS calcd for $C_{13}H_{17}O_6N_5$ (M - H + 2K) 416.0375, found 416.0356. λ_{max} = 265 nm.

***N*^{6}-(1-(S)-carboxy-2-(methylthio)ethyl)2'-deoxyadenosine (5h).** ^{1}H NMR (D_2O, 300 MHz): δ 1.97 ppm (s, 3H, CH_3), 2.35-2.46 (m, 1H, H2'), 2.65-2.73 (m, 1H, H2'), 2.89-3.08 (m, 2H, CH_2S), 3.58-3.70 (m, 2H, H5'), 4.02 (br s, 1H, H4'), 4.48 (br s, 1H, H3'), ~4.75 (under DHO, α-H), 6.32 [dd (6.4, 7.1 Hz), 1H, H1'], 8.10 (s, 1H, H8), 8.14 (s, 1H, H2). λ_{max} = 267 nm.

N^6-(1-(S)-carboxy-3-(methylthio)propyl)2'-deoxyadenosine (5i). ^{1}H NMR (D_2O, 300 MHz): δ 1.94 ppm (s, 3H, C*H*$_3$), 1.95-2.20 (m, 2H, C*H*$_2$CH$_2$S), 2.35-2.45 (m, 1H, H2'), 2.51 (br s, 2H, CH$_2$C*H*$_2$S), 2.63-2.75 (m, 1H, H2'), 3.58-3.72 (m, 2H, H5'), 4.03 (br s, 1H, H4'), 4.50 (br s, 1H, H3'), ~4.75 (under DHO, α-H), 6.32 [dd (~8 Hz), 1H, H1'], 8.08 (s, 1H, H8), 8.13 (s, 1H, H2). HRMS calcd for $C_{15}H_{21}O_5N_5S$ (M - H + 2K) 460.0460, found 460.0472. λ_{max} = 267 nm.

(N^6-(1-(S)-carboxy-5-aminopentyl)2'-deoxyadenosine (5j). ^{1}H NMR (D_2O, 300 MHz): δ 1.35-1.46 ppm (m, 2H, γ-C*H*$_2$ of amino acid), 1.55-1.68 (m, δ-C*H*$_2$), 1.76-1.93 (m, H, β-C*H*$_2$), 2.37-2.50 (m, 1H, H2'), 2.66-2.77 (m, 1H, H2'), 2.82-2.92 (m, 2H, ε-C*H*$_2$), 3.67 (br s, 2H, H5'), 4.05 (br s, 1H, H4'), 4.52 (2 br s, 2H, H3' and α-C*H*), 6.34 (br s, 1H, H1'), 8.10-8.20 (2 br s, 2H, H8 and H2). HRMS calcd for $C_{16}H_{24}O_5N_6$ (M + H) 381.1887, found 381.1905. λ_{max} = 265 nm.

N^6-(5-(S)-carboxy-5-aminopentyl)2'-deoxyadenosine (5k). ^{1}H NMR (D_2O, 300 MHz): δ 1.32-1.44 ppm (m, 2H, γ-C*H*$_2$), 1.55-1.69 (m, 2H, δ-C*H*$_2$), 1.72-1.85 (m, 2H, β-C*H*$_2$), 2.38-2.50 (m, 1H, H2'), 2.62-2.76 (m, 1H, H2'), 3.43 (br s, 2H, ε-C*H*$_2$), 3.58-3.74 (m and br s, 3H, H5' and α-C*H*), 4.04 (br s, 1H, H4'), 4.50 (br s, 1H, H3'), 6.32 (br s, 1H, H1'), 8.06 (br s, 1H, H8), 8.10 (br s, 1H, H2). HRMS calcd for $C_{16}H_{24}O_5N_6$ (M + H) 381.1887, found 381.1883. λ_{max} = 264 nm.

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5. The closely related ester 3',5'-diisobutyrl-O^6-(triisopropyl-benzenesulfonyl)-2'-deoxyinosine has been prepared by Seela, F., Herdering, W., Kehne, A., *Helv. Chim. Acta* **1987**, *70*, 1649.

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7. For each stability test, a few mg of O^6-aryl-2'-deoxyinosine were subjected to the following conditions: (1) 0.5 ml of 0.1 M I_2 in 1:10:40 water-lutidine-THF for 5 minutes. The reaction was quenched with sodium bisulfite and the product extracted out with methylene chloride. (2) 0.5 ml 3% dichloroacetic acid in methylene chloride for 30 minutes at room temperature. Product was isolated from the organic layer after neutralization of the reaction mixture with triethylamine and extraction with water. (3) 0.5 ml 0.5 M triazole in acetonitrile for 10 minutes. Concentrating the reaction mixture, taking up the residue in ethyl acetate, and extracting to neutrality with water allowed isolation of product from the organic layer.

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