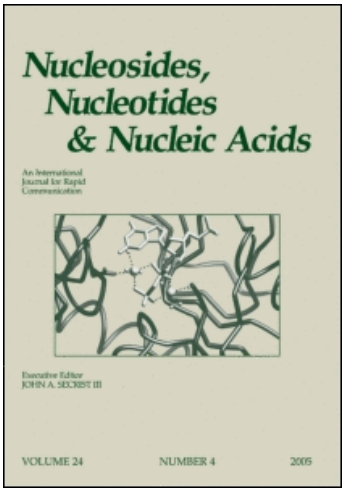

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A Rapid, High-Yield Method for 5'-Hydroxyl Protection in Very Reactive and Amino Group Modified Nucleosides Using Dimethoxytrityl Tetrafluoroborate

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A RAPID, HIGH-YIELD METHOD FOR 5'-HYDROXYL PROTECTION IN VERY REACTIVE AND AMINO GROUP MODIFIED NUCLEOSIDES USING DIMETHOXYTRITYL TETRAFLUOROBORATE

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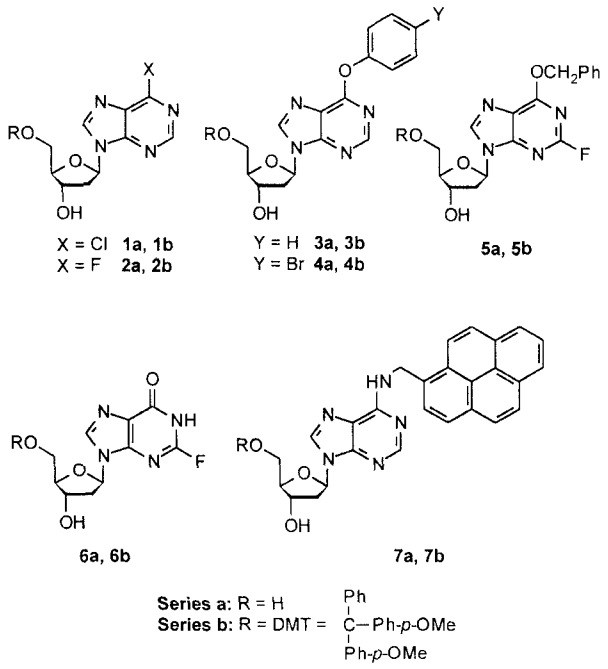
Abstract: The conventional method for 4,4'-dimethoxytrityl (DMT) etherification of the 5'-hydroxyl termini in deoxynucleosides that are either highly reactive or those bearing modifications at the exocyclic amino group can be quite problematic, and in several cases the yields are at best mediocre. Herein, we report a rapid, convenient and general procedure for the facile 5'-hydroxyl protection of these nucleosides in exceptionally high yields using $DMT^+BF_4^-$. This reagent is particularly useful for the preparation of 5'-*O*-DMT ethers of nucleosides that are either synthetically valuable or for those that undergo degradation under standard conditions.

DNA oligomers containing modified bases are valuable probes in the understanding of various biological processes.[1,2] At the present time there are primarily two methods for achieving such site-specific modification of DNA. The first is the more conventional approach where the base-modified nucleoside is directly incorporated by DNA synthesis.[2] The second approach, which is becoming increasingly popular, is a post-oligomerization modification strategy.[2-5] This method involves the incorporation of a reactive nucleoside that bears a leaving group (electrophile) into a DNA fragment. Subsequent reaction of the polymer-bound oligomer with nucleophiles results in modification of a specific base. In relation to our work in the area of carcinogen-DNA interactions we have been interested in both approaches to DNA modification.[6-9] However, we have encountered difficulties in the introduction of the 4,4'-dimethoxytrityl (DMT) moiety at the 5'-hydroxyl termini of nucleosides that are substantially modified at the exocyclic nitrogens as well as those of very reactive electrophilic nucleosides. In addition to our own experience, examples in the literature indicate that direct DMT



Figure

protection of reactive nucleosides such as **2a** and **6a** under standard conditions proceeds only in low yield (27% and 44%, respectively).[5,10] In another report the overall yield for a three-step conversion of **1a** to **2b** was only 16%.[11] The low yields in these cases may be partly due to competing side-reactions with the reaction solvent pyridine, which is nucleophilic. In our own experience a solution of **2a** in pyridine-d_5 turns red upon standing at room temperature for 24 hours. Although we have been unable to characterize a pyridinium salt, displacement of leaving groups from purines is a well known phenomenon.[12,13]

In principle, ideal conditions for achieving 5'-*O*-DMT protection of electrophilic nucleosides should exclude nucleophiles. Recently, the synthesis of 4,4'-dimethoxytrityl tetrafluoroborate ($DMT^+BF_4^-$) has been reported.[14] Given the nature of this salt, the reaction conditions for its application must also be devoid of nucleophiles, a feature that makes it particularly compatible with electrophilic nucleosides. Further, the high reactivity of $DMT^+BF_4^-$ might allow for facile reaction with other highly modified nucleosides. Although we have occasionally used $DMT^+BF_4^-$ in the past, the reaction conditions were far from optimal;[7,8] (a) substantial excess of the reagent was needed to ensure complete conversion, which posed the threat of undesired 3'-hydroxyl protection

and (b) removal of 2,6-lutidine, the reaction solvent, posed problems. Hence, an optimal set of conditions were required for the 5'-hydroxyl etherification with $DMT^+BF_4^-$. In this study, we disclose for the first time, a general application of $DMT^+BF_4^-$ for nucleoside protection. Under conditions we describe herein, very rapid reactions are observed with extremely high product yields in the cases of reactive and amino group modified nucleosides shown in the Figure.

RESULTS AND DISCUSSION

Preparation of the nucleoside substrates and improved preparation of O^6-substituted 2'-deoxyinosine derivatives

The halogenated 2'-deoxypurine nucleosides **1a** and **2a** were prepared as described by Robins and Basom.[15] The 2-fluoro-2'-deoxyinosine derivatives **5a** and **6a** were prepared by the method of Zajc *et al.*[8] Two separate syntheses of O^6-phenyl-2'-deoxyinosine (**3a**) have been reported with overall yields of 29% (three steps from 2'-deoxyinosine-3',5'-bis-*O*-acetate) and 42% (four steps beginning from 2'-deoxyadenosine).[11,16] As an alternative to these procedures, we have conveniently prepared phenoxy derivative (**3a**) and the 4-bromo analog (**4a**) from **1a**. Our three-step route beginning from 2'-deoxyinosine has overall yields >66% (the yields for the conversion of **1a** directly to either **3a** or **4a** are >90%, see the experimental section for details). This therefore, constitutes a substantial improvement both in yields and convenience in preparation of such phenoxy derivatives. Synthesis of the N^6-modified deoxyadenosine analog **7a** has been reported in the literature.[17] However, we have prepared **7a** by a condensation of **1a** with aminomethylpyrene,[18] where the latter compound has been synthesized via a newly reported conversion of benzylic alcohols to azides.[19]

Evaluation of the compatibility of solvent and proton sponge with $DMT^+BF_4^-$

Based on the nature of $DMT^+BF_4^-$, as Bleasdale *et al* recognized,[14] we needed a solvent and proton-sponge both of which do not coordinate with the salt. For our purposes based upon solubility considerations THF seemed to be the solvent of choice, and by prior experience[7,8] we decided to retain 2,6-lutidine as the hindered base. However, it was unclear whether coordination with THF would diminish the reactivity of $DMT^+BF_4^-$. To test this at the outset of our experimentation, a suspension of $DMT^+BF_4^-$ in THF and 2,6-lutidine (1 equivalent) was stirred at room temperature for 2 days. The orange precipitate of the salt persisted indicating that neither THF nor 2,6-lutidine was detrimental to the reactivity of $DMT^+BF_4^-$.[20] Further, the anticipated use of 2,6-lutidine in these reactions necessitated the development of a suitable workup procedure that would allow for its facile removal. It has been documented that nucleoside 5'-*O*-DMT ethers are stable to extractions with aqueous 10% citric acid.[21]

Optimization of conditions for the introduction of the DMT unit

With this initial information, we decided to investigate conditions for the preparation of **1b**. Thus, **1a** upon reaction with 1.1 equivalents of $DMT^+BF_4^-$ in THF and 5 equivalents of 2,6-lutidine led to complete consumption of the starting material within 1 h. Dilution of the reaction mixture with EtOAc, sequential extraction with water, 10% aqueous citric acid and saturated aqueous $NaHCO_3$[22] followed by chromatography afforded **1b** in 60-70% yield (lit. 70%[5]). Based upon this initial result we questioned whether complete removal of the formed weakly acidic 2,6-lutidinium tetrafluoroborate from the reaction medium was essential for yield improvement.[23] Therefore, when the conversion was repeated with two equivalents of added Li_2CO_3, complete consumption of **1a** was observed within 1 h and **1b** was isolated in 92-95% yield. Thus, neutralization of 2,6-lutidinium tetrafluoroborate by Li_2CO_3 seems to be a critical factor in these reactions.

Encouraged by these results we decided to apply this procedure to the preparation of the 5'-*O*-DMT ethers of phenoxy compounds **3a** and **4a**. When subjected to the tritylation conditions described above **3a** gave **3b** in 80% yield consistently (lit. 79%[3c]) while **4a** provided **4b** in 98% yield. With the results obtained from these relatively stable, less-reactive compounds the applicability of the method to the highly reactive fluoro nucleoside **2a** was tested. Under our reaction conditions protected **2b** was obtained in 91-94% yield.[24] Since this procedure allows for direct protection of **2a** in high-yield it obviates the need for less direct methods to **2b**.[10] At this stage we decided to evaluate a more hindered proton-sponge, 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP), in place of 2,6-lutidine. The reaction of **2a** with 1.2 equivalents each of $DMT^+BF_4^-$, DTBMP and 2 equivalents of Li_2CO_3 in THF was incomplete even after a prolonged period. However, the isolated yield of **2b** in one such reaction after 1 h (74%) was still significantly better than the literature yields. Thus, under our conditions and stoichiometry DTBMP seems to be less a effective proton-sponge than 2,6-lutidine.

Preparation of the 5'-*O*-DMT ether of 2-fluoro-2'-deoxyinosine (**6a**) presented an entirely new challenge. Attempts at a direct protection of **6a** provided **6b** in only 57% yield.[24] However, our observation, based on examples in the literature, indicated that O^6-protected 2-fluoro-2'-deoxyinosine derivatives can be converted to the 5'-*O*-DMT ethers in good yields.[25,26] Therefore, we reasoned that application of our procedure to the O^6-benzyl derivative **5a**,[8] followed by a mild hydrogenolysis of the benzyl protecting group should provide an overall yield improvement. Indeed as expected, when subjected to the protection conditions described **5a** afforded **5b** in 84% yield. At this stage a new problem was encountered during the course of the debenzylation of **5b**. Preliminary experiments indicated that debenzylation with 5% Pd-C was accompanied by some loss of the DMT group (as visualized on TLC), probably due to the activity of charcoal. This could be

Table: Best yields, TLC conditions[a,b] and R_f values for the DMT derivatives.

Cpd	% Yield	TLC eluting solvent	R_f
1b	95	EtOAc/Et_3N 99:1	0.21
2b	94	CH_2Cl_2/MeOH/Et_3N 95:4.5:0.5	0.48
		EtOAc/Et_3N 99:1	0.24
3b	80	CH_2Cl_2/MeOH/Et_3N 95:4.5:0.5	0.34
4b	98	CH_2Cl_2/MeOH/Et_3N 95:4.5:0.5	0.45
5b	84	EtOAc/hexane/Et_3N 80:19.5:0.5	0.32
6b	98[c]	CH_2Cl_2/MeOH/Et_3N 90:9.5:0.5	0.36
7b	94	CH_2Cl_2/MeOH/Et_3N 95:4.5:0.5	0.46

[a] Analytical TLC was performed using Analtech GHLF silica plates, 250 μ (2.5 x 10 cm).
[b] These systems do not necessarily reflect the solvent composition used for the purification of the products. Please see experimental section for details.
[c] This is the yield for the conversion of **5b** to **6b**.

supressed by addition of 2 equivalents of 2,6-lutidine to the reaction mixture, and no decrease in the reaction rate was observed indicating that the catalyst was not poisoned by the added base. However, due to the polar nature of **6b** severe problems were encountered during workup while attempting extractive removal of 2,6-lutidine. We found that these problems could be entirely circumvented by pretreating the catalyst with 2% 2,6-lutidine in MeOH.[27] Use of this catalyst led to smooth debenzylation of **5b** and afforded **6b** in 98% yield. It is worth noting that our procedure for the preparation of **6b** from **5a** does not involve any additional steps over the reported method.[5] It is simply a reversal in the order of events in the synthetic strategy combined with our newly developed method. Further, this overall approach nearly doubles the yield of protected 2-fluoro-2'-deoxyinosine **6b**.

As a final test of generality, we have applied this method to the preparation of the 5'-*O*-DMT ether of a nucleoside that contains a large hydrocarbon appended to the exocyclic amino group of deoxyadenosine. In the past we have encountered substantial difficulties in the DMT etherification of such adducted nucleosides. Nucleoside **7a** is structurally related to adducts produced upon DNA binding of the metabolically activated form of benzo[*a*]pyrene, a potent, environmentally prevalent carcinogen. Adduct **7a** when subjected to the tritylation conditions (using 1.3 equivalents of $DMT^+BF_4^-$) provided **7b** in 94% yield. For convenience, yields, tlc solvents and R_f values are shown in the Table.

In addition to the high yields in these reactions, it is equally important to point out another feature; the rapid reaction rates. This factor as well as the non-nucleophilic nature of the proton-sponge and solvent possibly contribute to the integrity of otherwise decomposable substrates. Thus, conventional tritylation conditions which may contribute to degradation of certain substrates can now be entirely avoided through the use of the presently described approach.

CONCLUSION

In this study, a reinvestigation of the dimethoxytrityl etherification has led to the development of a convenient, efficient, and general procedure for the introduction of the 5'-*O*-DMT group into very reactive and highly modified nucleosides. These reactions are extremely rapid in THF with near stoichiometric amounts of $DMT^+BF_4^-$ in the presence of 2,6-lutidine and Li_2CO_3. Thus, this set of reagents is a powerful combination that is capable of functionalizing 5'-hydroxyl groups in nucleosides that are normally difficult to convert to the corresponding DMT ethers. The significant yield improvements obtained through this approach are especially important in light of the multiple steps required for the preparation of modified nucleosides. Additionally, this method should be readily applicable to other exocyclic amino group modified nucleosides such as nucleoside adducts of the metabolically activated forms of environmentally prevalent carcinogens.

EXPERIMENTAL SECTION

Anhydrous THF and 2,6-lutidine were purchased from Aldrich and used without further purification. $DMT^+BF_4^-$ was prepared according to the literature procedure[14] and stored in a desiccator at 0 °C. In every case preparative chromatography was performed using TLC plates that had been predeveloped with the eluting solvent. Proton NMR spectra were recorded on a GE QE-300 (300 MHz) instrument. Chemical shifts (δ) are reported in ppm and coupling constants (*J*) are in Hz. High-resolution mass spectra were obtained on a VG Autospec-Q instrument. Products were homogenous as visualized on thin layer chromatography and were determined to be pure based upon their ^1H NMR spectra.

Typical procedure for the preparation of the O^6-phenyl-2'-deoxyinosine derivatives

O^6-(4-bromophenyl)-2'-deoxyinosine (4a):

A solution of **1a** (0.15 g, 0.56 mmol) and 4-bromophenol (0.596 g, 3.44 mmol) in 1,2-dimethoxyethane (5 mL) was cooled to 0 °C. Me_3N was bubbled through the mixture for 10 min, Et_3N (0.27 mL, 1.94 mmol) was added and the mixture was stirred at 0 °C for 1 h. DMF (1 mL) was added, the mixture was allowed to warm to room temperature and stirred for 2 h at which time the reaction was complete. The mixture was evaporated to

dryness and the crude product mixture was loaded onto a silica gel column packed in CH_2Cl_2. Excess 4-bromophenol was removed by elution with CH_2Cl_2, subsequent elution with 5% MeOH in CH_2Cl_2 afforded **4a** as a colorless powder (0.21 g, 92%). TLC (CH_2Cl_2/MeOH 9:1) R_f = 0.38. ^{1}H NMR ($CDCl_3$): 8.49, 8.10 (2s, $2H_{2,8}$); 7.59 (d, $2H_{Ar}$, J = 8.7); 7.17 (d, $2H_{Ar}$, J = 8.7); 6.43 (dd, $1H_{1'}$, J = 5.5, 9.5); 5.84 (dd, $1H_{5'\text{-}OH}$, J = 1.6, 11.3); 4.83 (br m, $1H_{3'}$); 4.26 (br s, $1H_{4'}$); 3.99 (d, $1H_{5'}$, J = 11.1); 3.82 (t, $1H_{5'}$, J_{app} = 11.3); 3.12 (m, $1H_{2'}$); 2.38 (dd, $1H_{2'}$, J = 5.5, 13.0); 1.95 (d, $1H_{3'\text{-}OH}$, J = 2.9).

O^6-Phenyl-2'-deoxyinosine (3a):

Prepared from **1a** (0.199 g, 0.737 mmol), phenol (0.43 g, 4.57 mmol), Me_3N, Et_3N (0.36 mL, 2.59 mmol), 1,2-dimethoxyethane (5 mL) and DMF (1 mL) in a manner similar to that described for the preparation of **4a**. TLC (CH_2Cl_2/MeOH 9:1) R_f = 0.52. This product was identical to that reported.[11,16] ^{1}H NMR ($CDCl_3$): 8.49, 8.09 (2s, $2H_{2,8}$); 7.20-7.51 (m, $5H_{Ar}$); 6.42 (dd, $1H_{1'}$, J = 5.4, 9.5); 5.96 (dd, $1H_{5'\text{-}OH}$, J = 1.7, 11.5); 4.82 (br m, $1H_{3'}$); 4.26 (br s, $1H_{4'}$); 3.99 (d, $1H_{5'}$, J = 11.5); 3.82 (t, $1H_{5'}$, J_{app} = 11.3); 3.12 (m, $1H_{2'}$); 2.38 (dd, $1H_{2'}$, J = 5.3, 13.3); 2.00 (d, $1H_{3'\text{-}OH}$, J = 2.9).

Typical procedure for the preparation of the 5'-O-DMT ethers

6-Fluoro-9-(2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)purine (2b):

6-Fluoro-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purine (**2a**) (33.2 mg, 0.131 mmol), $DMT^+BF_4^-$ (61.2 mg, 0.157 mmol) and Li_2CO_3 (19.3 mg, 0.261 mmol) were dried *in vacuo* for 1 h. 2,6-Lutidine (76.1 μL, 0.656 mmol) followed by THF (0.5 mL) were added and the mixture was stirred at room temperature for 1 h. The mixture was then diluted with EtOAc and washed sequentially with H_2O, 10% aq. citric acid and saturated aq. $NaHCO_3$ (twice). The organic layer was dried over Na_2SO_4 and evaporated to leave a yellow product. Chromatography on a preparative silica plate (1 mm, 20 x 20 cm) using CH_2Cl_2/MeOH/Et_3N 98:1.5:0.5 afforded product **2b** as an off-white powder. ^{1}H NMR ($CDCl_3$): 8.56, 8.25 (2s, $2H_{2,8}$); 7.40-7.20 and 6.82-6.79 (13H, aromatic); 6.52 (t, $1H_{1'}$, J = 6.4); 4.72 (m, $1H_{3'}$); 4.18 (app q, $1H_{4'}$ J_{app} ~ 4); 3.79 (s, 6H, OCH_3); 3.45 (dd, $1H_{5'}$, J = 4.5, 10.2); 3.39 (dd, $1H_{5'}$, J = 4.8,10.2); 2.88 (app quint, $1H_{2'}$, J_{app} = 6.4); 2.61 (ddd, $1H_{2'}$, J = 4.4, 6.4, 13.4). HRMS calcd. for $C_{31}H_{30}FN_4O_5$ (M+1) 557.2200, found 557.2186.

O^6-Benzyl-2-fluoro-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyinosine (5b):

The reaction was performed essentially as described for the preparation of **2b** using O^6-benzyl-2-fluoro-2'-deoxyinosine[8] (**5a**) (32 mg, 88.89 μmol), $DMT^+BF_4^-$ (41.6 mg, 0.107 mmol), Li_2CO_3 (13.1 mg, 0.177 mmol) and 2,6-lutidine (52 μL, 0.448 mmol)

in THF (0.5 mL). Chromatography of the crude product on a preparative silica plate (1 mm, 20 x 20 cm) using EtOAc/hexane/Et_3N 70:29.5:0.5 afforded product **5b** as pale yellow powder. ^1H NMR ($CDCl_3$): 8.02 (s, $1H_8$); 7.55-7.20 and 6.87-6.81 (18H, aromatic); 6.38 (t, $1H_{1'}$, J = 6.5); 5.64 (s, 2H, OCH_2); 4.65 (br m, $1H_{3'}$); 4.13 (app q, $1H_{4'}$, J_{app} ~ 4); 3.77 (s, 6H, OCH_3); 3.45 (dd, $1H_{5'}$, J = 4.7, 10.2); 3.35 (dd, $1H_{5'}$, J = 4.9, 10.2); 2.78 (app quint, $1H_{2'}$, J_{app} = 6.4); 2.52 (ddd, $1H_{2'}$, J = 4.2, 6.4, 13.4). HRMS calcd. for $C_{38}H_{36}FN_4O_6$ (M+1) 663.2619, found 663.2606.

2-Fluoro-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyinosine (6b):

To a stirred suspension of pretreated 5% Pd-C[27] in 1:1 THF-MeOH (2 mL) was added the 5'-*O*-DMT protected O^6-benzyl-2-fluoro-2'-deoxyinosine **5b** (46.4 mg, 70.1 μmol). The mixture was stirred in a hydrogen atmosphere (using a balloon) at room temperature for 30 min and filtered through Celite which had been prewashed with 0.5% Et_3N in MeOH. The residue was washed with THF, the filtrate was evaporated to leave product **6b** as a colorless solid, which was finally washed with a minimum volume of cold Et_2O and collected by centrifugation. ^1H NMR (acetone-d_6): 8.05 (s, $1H_8$); 7.44-7.15 and 6.83-6.78 (13H, aromatic); 6.34 (t, $1H_{1'}$, J = 6.4); 4.63 (m, $1H_{3'}$); 4.14 (m, $1H_{4'}$); 3.75 (s, 6H, OCH_3); 3.38 (dd, $1H_{5'}$, J = 5.6, 10.2); 3.28 (dd, $1H_{5'}$, J = 4.0, 10.2); 2.82 (app quint, $1H_{2'}$, J_{app} = 6.4); 2.47 (ddd, $1H_{2'}$, J = 4.2, 6.4, 13.4). HRMS calcd. for $C_{31}H_{30}FN_4O_6$ (M+1) 573.2149, found 573.2157.

6-Chloro-9-(2-deoxy-5-*O*-(4,4'-dimethoxytrityl)-β-D-*erythro*-pentofuranosyl)purine (1b):

Prepared from **1a** (30.7 mg, 0.114 mmol), $DMT^+BF_4^-$ (53.2 mg, 0.136 mmol), 2,6-lutidine (66 μL, 0.57 mmol), Li_2CO_3 (16.8 mg, 0.227 mmol) in THF (0.5 mL). Conditions for preparative TLC: EtOAc/Et_3N 99:1. ^1H NMR ($CDCl_3$): 8.67, 8.28 (2s, $2H_{2,8}$); 7.39-7.24 and 6.82-6.79 (13H, aromatic); 6.50 (t, $1H_{1'}$, J = 6.4); 4.72 (m, $1H_{3'}$); 4.18 (app q, $1H_{4'}$ J_{app} ~ 4); 3.79 (s, 6H, OCH_3); 3.45 (dd, $1H_{5'}$, J = 4.5, 10.3); 3.39 (dd, $1H_{5'}$, J = 5.0, 10.3); 2.88 (app quint, $1H_{2'}$, J_{app} = 6.3); 2.60 (ddd, $1H_{2'}$, J = 4.2, 6.4, 13.5). HRMS calcd. for $C_{31}H_{30}ClN_4O_5$ (M+1) 573.1905, found 573.1914.

O^6-Phenyl-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyinosine (3b):

Prepared from **3a** (31.4 mg, 95.7 μmol), $DMT^+BF_4^-$ (44.8 mg, 0.115 mmol), 2,6-lutidine (55.7 μL, 0.48 mmol), Li_2CO_3 (14.1 mg, 0.19 mmol) in THF (0.5 mL). Conditions for preparative TLC: CH_2Cl_2/MeOH/Et_3N 96.5:3:0.5. ^1H NMR ($CDCl_3$): 8.44, 8.17 (2s, $2H_{2,8}$); 7.49-7.26 and 6.82-6.79 (18H, aromatic); 6.51 (t, $1H_{1'}$, J = 6.4); 4.72 (m, $1H_{3'}$); 4.16 (app q, $1H_{4'}$ J_{app} ~ 4); 3.79 (s, 6H, OCH_3); 3.46 (dd, $1H_{5'}$, J = 4.7, 10.2); 3.39 (dd, $1H_{5'}$, J = 5.1, 10.2); 2.89 (app quint, $1H_{2'}$, J_{app} = 6.3); 2.59 (ddd, $1H_{2'}$, J = 4.5, 6.4, 13.4). HRMS calcd. for $C_{37}H_{35}N_4O_6$ (M+1) 631.2557, found 631.2572.

***O*^{6}-(4-Bromophenyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyinosine (4b):**

Prepared from **4a** (31.0 mg, 76.4 μmol), $DMT^+BF_4^-$ (35.7 mg, 91.5 μmol), 2,6-lutidine (44.5 μL, 0.383 mmol), Li_2CO_3 (11.3 mg, 0.153 mmol) in THF (0.5 mL). Conditions for preparative TLC: CH_2Cl_2/MeOH/Et_3N 96.5:3:0.5. ^1H NMR ($CDCl_3$): 8.43, 8.18 (2s, $2H_{2,8}$); 7.52-7.15 and 6.82-6.79 (17H, aromatic); 6.51 (t, $1H_{1'}$, J = 6.4); 4.72 (m, $1H_{3'}$); 4.16 (app q, $1H_{4'}$ J_{app} ~ 4); 3.78 (s, 6H, OCH_3); 3.46 (dd, $1H_{5'}$, J = 4.7, 10.1); 3.39 (dd, $1H_{5'}$, J = 5.0, 10.1); 2.90 (app quint, $1H_{2'}$, J_{app} = 6.3); 2.59 (ddd, $1H_{2'}$, J = 4.5, 6.5, 13.6). HRMS calcd. for $C_{37}H_{34}BrN_4O_6$ (M+1) 709.1662, found 709.1664.

***N*^{6}-(Methylpyrenyl)-5'-*O*-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (7b):**

Prepared from **7a** (21 mg, 45.2 μmol), $DMT^+BF_4^-$ (23 mg, 59 μmol), 2,6-lutidine (26.3 μL, 0.227 mmol), Li_2CO_3 (6.7 mg, 90.7 μmol) in THF (0.5 mL). Conditions for preparative TLC: CH_2Cl_2/MeOH/Et_3N 95:4.5:0.5. ^1H NMR ($CDCl_3$): 8.44-6.77 (25H, 2, 8, aromatic, N*H*); 6.39 (t, $1H_{1'}$, J = 6.3); 5.57 (br s, 2H, CH_2); 4.66 (m, $1H_{3'}$); 4.11 (br q, $1H_{4'}$); 3.74 (s, 6H, OCH_3); 3.39 (m, $2H_{5'}$); 2.78 (app quint, $1H_{2'}$, J_{app} = 6.3); 2.50 (ddd, $1H_{2'}$, J = 4.3, 6.3, 13.3). HRMS calcd. for $C_{48}H_{42}N_5O_5$ (M+1) 768.3186, found 768.3188.

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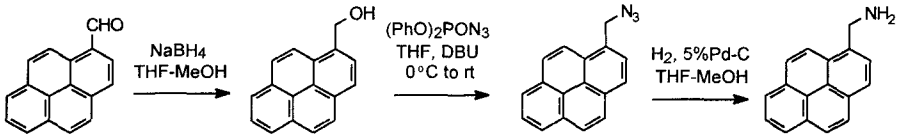
18. Compound **7a** was prepared as follows: aminomethylpyrene[19] (0.119 g, 0.515 mmol) and **1a** (0.153 g, 0.567 mmol) were heated with Et_3N (0.21 mL, 1.51 mmol) in DMF (2 mL) at 50-55 °C for 16 h. Another portion of **1a** (27.7 mg, 0.103 mmol) was added and the heating was continued for 5 h. The mixture was cooled, diluted with EtOAc, washed with water and brine. The aqueous layers were extracted once with EtOAc. The combined organic layer was dried over Na_2SO_4 and evaporated. The product was dissolved in 2:1 EtOAc-MeOH with a small volume of added DMF, slurried with silica gel and evaporated to dryness. The product impregnated silica gel was loaded onto a short dry-packed silica gel column. Sequential elution with EtOAc and 5% MeOH in EtOAc afforded the adduct as a colorless powder which was washed with Et_2O (0.156 g, 65%). TLC (CH_2Cl_2/MeOH 9:1) R_f= 0.39.
19. Although the preparation of aminomethylpyrene has been reported in the literature (ref. 17), we have prepared this compound through the route shown below. The key

conversion of pyrenemethanol to azidomethylpyrene was accomplished based on a report describing a direct conversion of benzylic alcohols to azides (ref. Thompson, A. S.; Humphrey, G. R.; DeMarko, A. M.; Mathre, D. J.; Grabowski, E. J. J. *J. Org. Chem.* **1993**, *58*, 5886-5888).



20. Consumption of $DMT^+BF_4^-$ results in the disappearance of the bright orange precipitate.
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22. The sodium bicarbonate wash during workup is essential to remove traces of citric acid from the organic layer. In one instance, during the preparation of **2b**, the bicarbonate wash was intentionally omitted. Subsequently, significant product decomposition occurred during evaporation of the organic layer as evidenced by appearance of the characteristic red color of the DMT cation.
23. Although no significant starting compound was observed by TLC at the end of the reaction 2,6-lutidinium tetrafluoroborate is acidic and in principle, can cause deprotection of the DMT ether.
24. Reaction of **2a** or **6a** with $DMT^+BF_4^-$ and Li_2CO_3 in 2,6-lutidine gives the 5'-*O*-protected products in yields comparable to those reported (refs. 5 and 10).
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26. See supplementary material to Erlanson, D. A.; Chen, L.; Verdine, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 12583-12584. Yield of the O^6-*p*-nitrophenylethyl analog of **5b**: 80%.
27. Pretreatment of the catalyst was performed as follows: 5% Pd on C (50 mg) was washed with 2% 2,6-lutidine in MeOH (2 x 2.5 mL). Traces of 2,6-lutidine were removed by washing with THF and the catalyst was dried *in vacuo*.

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