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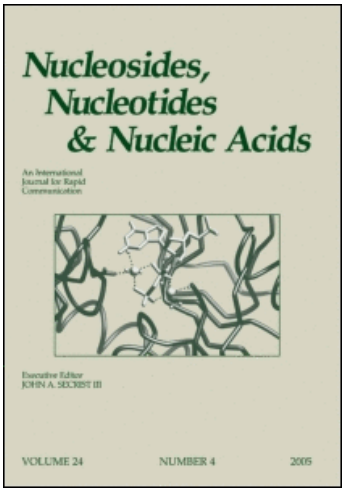

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Synthesis of 8-Bromo- and 8-Azido-2'-deoxyadenosine-5'-O-(1-thiotriphosphate)

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NUCLEOSIDES & NUCLEOTIDES, 12(7), 757-771 (1993)

SYNTHESIS OF 8-BROMO- AND 8-AZIDO-2'-DEOXYADENOSINE-5'-O-(1-THIOTRIPHOSPHATE)

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ABSTRACT

Treatment of 3'-O-methoxyacetylated 8-bromo-2'-deoxyadenosine (**5**) with a twofold excess of salicyl phosphorochloridite (**6**) and subsequent reaction with bis(tri-*n*-butylammonium) pyrophosphate and oxidation wih sulfur followed by removal of the protecting group gives predominantly 8-bromo-2'-deoxyadenosine-5'-O-(1-thiotriphosphate) (**7**) and minor amounts of the corresponding brominated monothiophosphate. Alternatively, the photoreactive dATP analog 8-azido-2'-deoxyadenosine-5'-O-(1-thiotriphosphate) (**11**) is obtained by phosphorylation of unprotected 8-azido-2'-deoxyadenosine (**9**) with a 1.8 molar equivalent excess of thiophosphoryl chloride and bis(tri-*n*-butylammonium) pyrophosphate. A protection of the nucleobase 6-amino group is not required. The photoaffinity labeling reagent **11** was characterized by ^{31}P-NMR and ion-spray mass spectroscopy and its photolysis upon long wavelength UV irradiation was studied. Both α-thioderivatives of 2'-deoxyadenosine triphosphates can be incorporated into plasmid DNA by T7 DNA polymerase. Thus, they can be used for interference studies of protein binding and for cross-linking with amino acids in protein-nucleic acid-complexes.

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INTRODUCTION

Nucleoside-α-thiotriphosphates are used as substrates for DNA and RNA polymerases as recently reviewed [1]. The substitution of a non-bridging oxygen at the α-phosphate group with sulfur confers chirality on this particular phophorus atom. Since nucleosides themselves are chiral, that leads to a pair of diastereomers. They have been employed to determine the stereochemical course of a large number of enzymatic reactions [1, 2, 3]. It is often found that thiophosphorylated nucleic acid alter the affinity of a binding protein. Data from such experiments were used to determine phosphate contacts that interfere with the R17 coat protein [4], as well as the location of specific phosphodiester bonds in the hammerhead ribozyme self-cleavage domain [5]. Phosphorothioate-containing DNA can be selectively cleaved by an alkylating reagent followed by hydrolysis. This property was recently exploited in a new method for sequencing RNA and DNA [6, 7]. Instead of cleaving the phosphorothioate diester, a number of different reporter groups can be incorporated site-specifically into modified DNA sequences [8]. Photoreactive azido nucleotide analogs have been applied to investigate specific interactions of DNA-binding proteins [9, 10] and enzymes [11, 12, 13] by photoaffinity labeling. Upon irradiation with long wavelength UV light [14], these compounds form highly reactive nitrenes that bind covalently to adjacent amino acids of the protein [15], allowing the labeled protein to be identified and characterized [16].

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We describe here the synthesis and characterization of the dATP analogs 8-bromo and 8-azido-2'-deoxyadenosine-5'-O-(1-thiotriphosphate) as a combination of a α-thio-triphospate and an 8-substituent at the purine ring in a single nucleotide derivative. Both compounds can be incorporated *in vitro* into plasmid DNA. Thus, they offer the possibility of combining interference and photo-crosslinking studies of sequence specific protein binding with the sequencing techniques based on α-thio-phosphodiesters in DNA [6].

RESULTS AND DISCUSSION

Our approach to synthesize the dATP derivatives 8-bromo and 8-azido-2'-desoxyadenosine-5'-O-(1-thiotriphosphate) made use of two different methods (Scheme 1). The concept of transient protection of the 5'- and 3'-hydroxyl group of 2'-deoxyadenosine (**1**) was applied to yield 8-bromo-2'-deoxyadenosine-5'-O-(1-thiotriphosphate) (7) (method A). Nucleoside **1** was converted into its 5'-O-4,4'-dimethoxytrityl (DMTr) derivative (**2**). The O-3' methoxyacetyl protecting group was introduced by the action of methoxyacetic anhydride in the presence of pyridine, forming compound **3**. Subsequent treatment of the fully protected derivative **3** with 80% acetic acid selectively removed the 4,4'-dimethoxytrityl group as reported [17], resulting in 3'-O-

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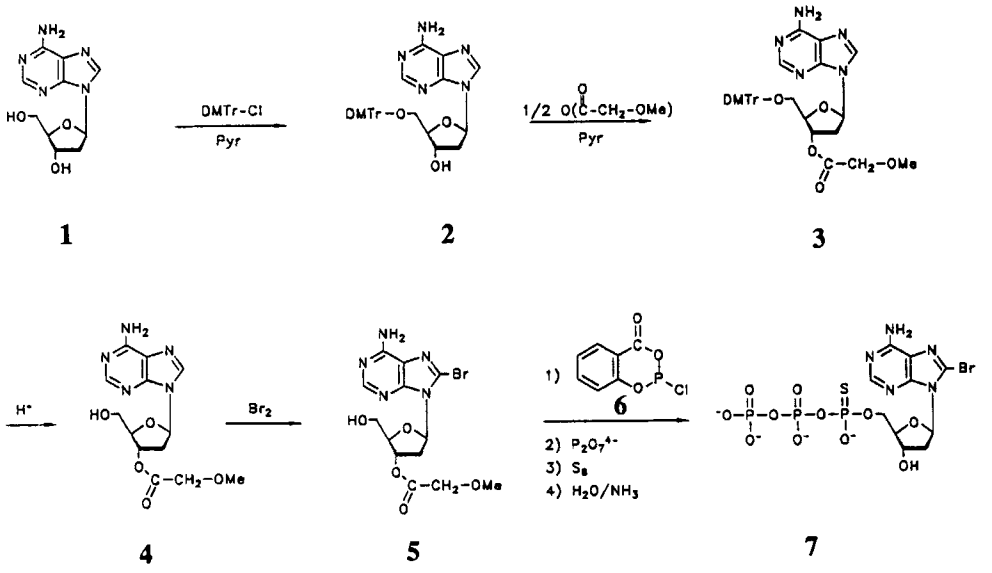
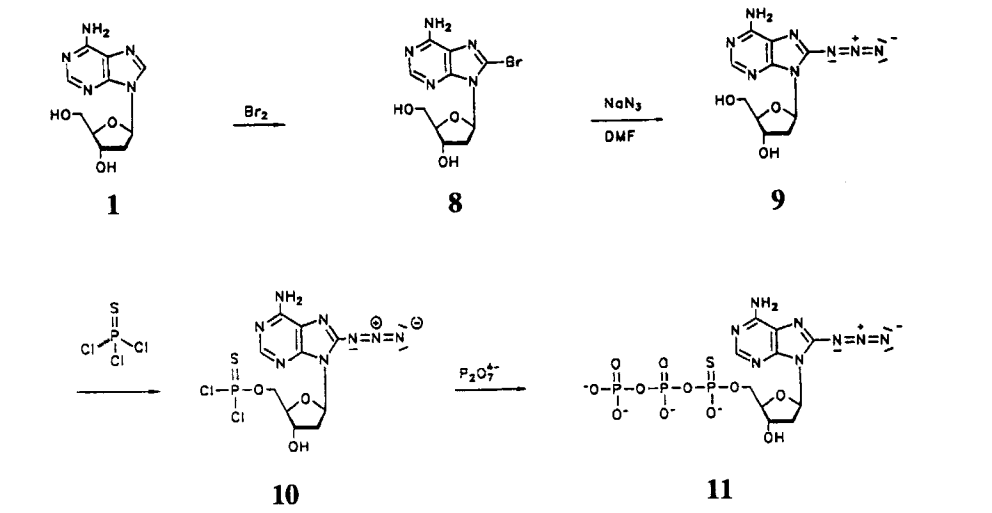
method A



1 2 3 4 5 7

method B



1 8 9 10 11

SCHEME 1

methoxyacetyl-2'-deoxyadenosine (**4**). Direct bromination of **4** in a mixture of dioxane and 10% Na_3PO_4 solution gave 8-bromo-3'-O-methoxyacetyl-2'-deoxyadenosine (**5**) in yields greater than 85%. Compound **5** was treated with salicyl phosphorochloridite (**6**) and bis(tri-*n*-butylammonium) pyrophosphate in a one-flask-reaction [18]. The intermediate was oxidized with sulfur and hydrolyzed, followed by deprotection of the O-3' hydroxyl group with ammonia, to give 8-bromo-2'-deoxyadenosine-(1-thiotriphosphate) (**7**).

The treatment of 8-bromo-3'-O-methoxyacetyl-2'-deoxyadenosine (**5**) with sodium azide in DMF did not result in the formation of the corresponding 8-azido-3'-O-methoxyacetyl-2'-deoxyadenosine. Besides replacement of the 8-bromo substituent the nucleophilic azide ion removed the labile methoxyacetyl group and yielded 8-azido-2'-deoxyadenosine (**9**) as proved by ^{1}H-NMR and a bathochromic shift from 265 to 280 nm in the UV absorbtion maximum. Bromination of dATPαS is known to result in oxidative removal of the sulfur atom [19]. The possibility of introducing the azido group via 8-bromo-2'-deoxyadenosine-(1-thiotriphosphate) (**7**) should give a mixture of the corresponding 8-N_3dAMPαS, 8-N_3dADPαS and 8-N_3dATPαS nucleotides as well as unreacted 8-bromo analogs. This was recently observed by Meffert and co-workers [10] and is based on the assumption of limited hydrolytic cleavage on the synthesis of 8-N_3dATP.

Instead of trying to find a useful protecting group, an alternative synthetic route was employed according to [20] (method B). The corresponding 8-azido nucleoside (**9**) was synthesized from **1** via 8-bromo-2'-deoxyadenosine (**8**). The first two reactions were accomplished without any chromatographic isolation of the products: nucleoside **8** could be easily separated from traces of unreacted starting material **1** by chloroform extraction in an overall yield of 80%. Compound **9** was isolated after reaction with sodium azide in DMF. After removal of the precipitated salts, brominated nucleoside **8** was separated from insoluble **9** in 87% yield with ethanol. The mixture of **9** with $PSCl_3$ in triethyl phosphate and 2,6-lutidine gave the corresponding thiophosphorodichloridate nucleoside intermediate (**10**). In a following reaction with bis(tri-*n*-butylammonium) pyrophosphate, the tri-*n*-butylammonium salt of 8-N_3dATPαS (**11**) was obtained after hydrolysis in triethylammonium bicarbonate (TEAB) buffer. Compound **11** was further purified by ion-exchange chromatography.

In both methods yields were lower than that of the previously reported synthesis of dNTPαS [18, 20], probably due to steric hindrance by the bulky 8-bromine atom in **5** and the 8-azido group in **9** with either salicyl phosphorochloridite or $PSCl_3$. Both compounds are reported to exist in the *syn* conformation as well as their corresponding α-thiotriphosphates, in contrast to the more usual *anti* conformation found in purine nucleotides [21] and nucleosides [22, 23]. A conformational analysis of **7** and **11** by force-

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field calculation resulted in a *syn* conformation as the lowest energetic state (data not shown).

Major by-products of both reactions, in addition to unreacted starting material, were the monophosphorothioate nucleotides that eluted on an anionic-exchange resin at about 0.2 M TEAB. This demonstrates that especially in method B the reaction between the intermediate thiophosphorodichloridate and pyrophosphate is the most critical step. A longer reaction time led to formation of higher polyphosphates as had been shown previously by Goody and Isakov on the synthesis of dNTPαS [20]. Moreover, to obtain partial dissolution it was necessary to heat compound **9** at 160° C for 2 min in triethyl phosphate. Even then, a fraction of compound **9** remained undissolved, while in another fraction the labile azido group was decomposed by heating. Other by-products were formed which were not identified. Purification of **7** and **11** was carried out on a column of Fractogel TSK DEAE using TEAB buffer at a pH of 7.9 as eluant. The triethylammonium salts of **7** and **11** were eluted between 0.35-0.4 M TEAB. The overall yields based on **1** were 36% for **7** and 5% for **11**. Both 8-substituted α-thiotriphosphates migrate slower on ion-exchange TLC than unsubstituted dATPαS and dATP, respectively. Their structures were characterized by ^{31}P-NMR, UV spectrometry and reversed-phase HPLC and compared with dATP and dATPαS (see Experimental and Table 1, 2) to unambiguously show that both modifications are introduced into the same nucleotide derivative. Furthermore, compound **11** was characterized by ion-spray mass spectrometry (ISMS) by direct injection to rule out degradation of the azido group. The analysis of the HPLC-purified and sodium salt exchanged dATP derivative is shown in Figure 1. Ions (m/z) of 636.8 and 614.6 correspond to the tetra- and trisodium salts of $(8\text{-}N_3dATP\alpha S)^+$ respectively. Another signal at (m/z) 460.1 is assigned to (Na_4-monothiotriphosphate-2'-deoxyadenosine)$^+$ formed by loss of the purine ring due to cleavage of the N-glycosylic bond. In the negative mode, the molecular ion peaks of **11** $(8\text{-}N_3dATP\alpha S)^-$ at (m/z) 546.4 for the free acid and for the monosodium salt at (m/z) 568.4 were obtained, as well as other fragments originating from the monothiotriphosphate group. All other major signals could be identified as fragments of **11**.

The combination of the chiral nucleoside moieties of **7** and **11** with the chiral α-phosphorus leads to a pair of S_p- and R_p-diastereomers. They could be analyzed by ^{31}P-NMR and compared to dATP (Table 1). These results are in agreement with those in previous reports dealing with α-thiotriphosphates [18, 20]. However, reversed-phase HPLC of the newly synthesized α-thiotriphosphate derivatives failed to resolve the individual diastereomers at different pHs (5. 6.7, 7.5 and 9). The increased hydrophobicity of either the azido or bromo substituent compared to dATP and dATPαS apparently accounts for the dramatic increase in retention times (Figure 2) and may mask any

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Table 1. ^{31}P-NMR Chemical Shifts and UV Spectral Data.

	chemical shifts, ppm[a]			UV[b] [nm]	
	P_α	P_β	P_γ	λ_{max}	λ_{min}
dATP	-10.6 (d)	-22.5 (m)	-10.2 (d)	259	226
dATPαS	42.96 (2d)	-23.89 (m)	-10.72 (d)	259	229
(7)	43.75 (2d)	-23.17 (m)	-8.05 (d)	265	232
(**11**)	44.10 (2d)	-23.60 (2d)	-10.20 (d)	282	249

[a]For ^{31}P-NMR conditions see Experimental.
[b]UV spectra were recorded in a 10 mM Tris buffer (pH 7.5).
Compounds 7, **11** and dATPαS were measured as a mixture of two diastereomeres.

Table 2. Chromatographic Data of dATP and Derivatives.

	HPLC[c] retention times t_R [min]	TLC[d] (R_f values)
dATP	13.53	0.36[e]
dATPαS	14.57 (S_p), 15.00 (R_p)	0.27[e]
(7)	16.99 (S_p + R_p)	0.13[f]
(**11**)	16.84 (S_p + R_p)	0.25[e]

[c]For instrumentation see Experimental; reversed-phase HPLC on a Vydac column (C_{18}, 5 μm) with 50 mM triethylammonium acetate, pH 6.7 (A) and acetonitrile (B) as eluant at a flow rate of 0.7 ml/min and a gradient of B in A (0 - 20 %) in 20 min. [d]PEI-Cellulose anionic-exchange plates; solvent: [e]0.6 M TEAB, pH 7.9; [f]solvent 0.4 M TEAB pH 7.7.

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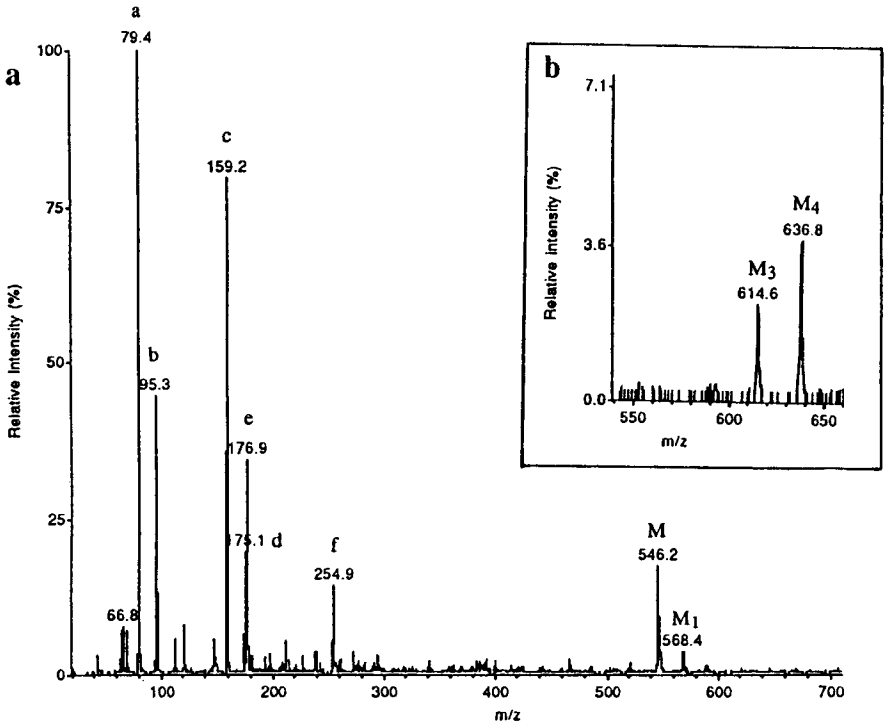

Figure 1. Ion-spray mass spectra of the modified nucleotide 8-N_3dATPαS **11** in acetonitrile. Negative mode (a). Positive mode (b). In the inset a more enlarged spectrum of the mass range at m/z 550 to 650 is depicted. a: $(PO_3)^-$, b: $(PSO_2)^-$, c: $(P_2O_6H_2)^-$, d: (8-N_3adenine-H)$^-$, e: $(P_2O_5SH_2)^-$, f: $(P_3O_8SH_3)^-$, M: (**11**-H)$^-$, M_1: (**11**-2H+Na)$^-$, M_3: (**11**-2H+3Na)$^+$, M_4: (**11**-3H+4Na)$^+$.

difference in association of the diastereomers to a reversed-phase support. HPLC of the nucleotide dATPαS shows two well-resolved peaks, which is in agreement with previous publications dealing with dNTPαS [2, 18, 20]. The ^{31}P-NMR spectroscopy of **7** and **11** (Table 1) clearly shows them as a mixture of two diastereomeric compounds that split into two dubletts (P_α). The elution profiles of 8-BrdATPαS **7** and 8-N_3dATPαS **11** and dATPαS and dATP as standards are depicted in Figure 2. The UV absorbtion spectrum of 8-N_3dATPαS (**11**) shows a maximun at 282 nm (see Table 1). Its photolysis in aqueous solution (10 mM Tris-HCl, pH 7.0) was followed spectroscopically (Figure 3). The occurence of isosbestic points indicates that the photolysis is largely controlled by a single of reaction which was recently suggested for 8-N_3dATP [10].

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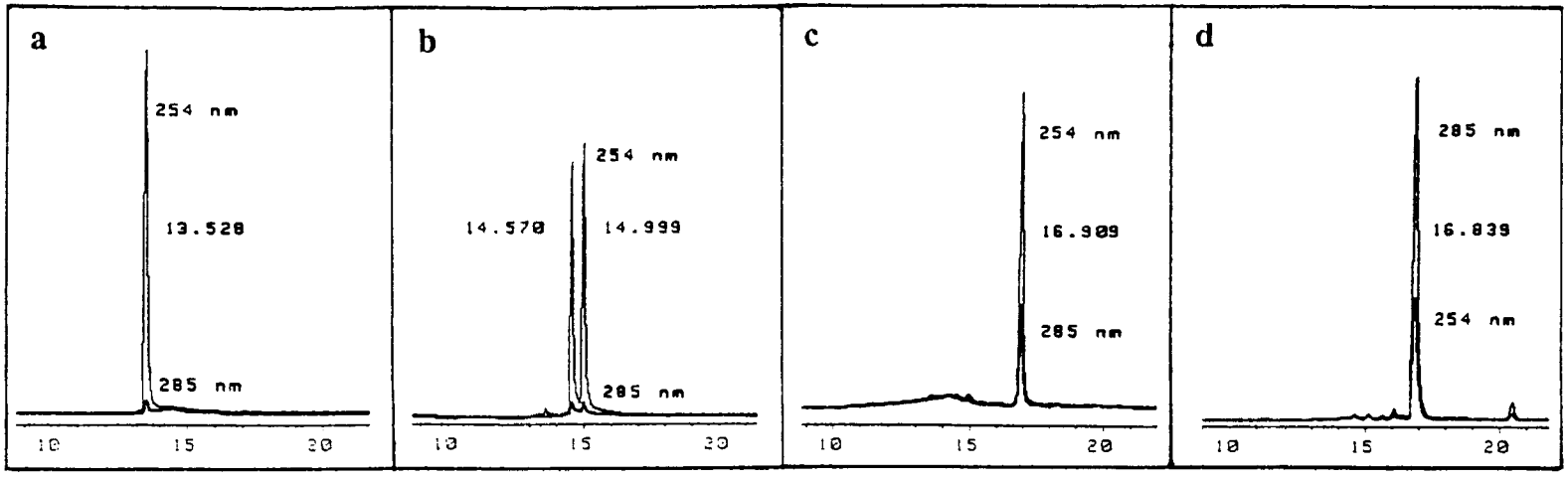

Figure 2. Reversed-phase HPLC chromatogramms of dATP (a), dATPαS (b), 7 (c) and **11** (d) monitored at 254 and 285 nm simultaneously. Conditions see Table 2 and Experimental.

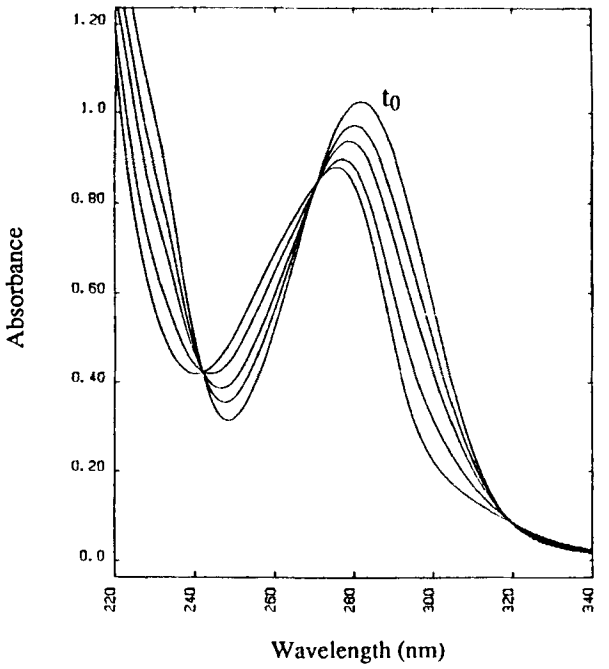

Figure 3. Photolysis of 8-N_3dATPαS in Tris buffer (10 mM Tris-HCl, pH 7.0) by irradiation with UV light (λ_{max} of lamp = 366 nm) at room temperature. Samples were irradiated in a quartz cuvette with a hand lamp at a distance of 3 cm for 0, 2, 4, 9 and 19 min.

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To prove that the newly synthesized nucleotide derivatives 7 and **11** are substrates for T7 DNA polymerase an enzymatic incorporation into a plasmid DNA was carried out. A mixture of dGTP, dTTP and [α-^{32}P]dCTP with either a 10 fold excess of the sodium-salts of compounds 7 or **11** were incubated with a primer annealed single-stranded DNA (ssmWH14). The double-stranded and labelled DNA was applied to a 1 % agarose gel and electrophoresed. Its autoradiograph (data not shown) demonstrated the incorporation of 8-BrdATPαS and 8-N_3dATPαS into DNA. The yield of incorporation was about 50% compared to the one of dATP and dATPαS.

The second method described here (method B) represents a rapid three step synthesis of 8-N_3dATPαS (**11**). It can be accomplished for the 8-BrdATPαS (7) as well as for other nucleotides. In the first step an overall yield greater than 80% for **8** was obtained, which is a significant improvement over the previously published procedures (66% for **8** [25]). The introduction of the monothiophosphate group into the 8-substituted nucleoside

is the most critical step. No further efforts to optimize yields were performed, but even the comparatively low yields produced enough material for a very large number of molecular biological experiments like the study of protein-DNA interactions.

EXPERIMENTAL

Thin-layer chromatography (HPTLC) was performed on either silica gel 60 F_{254} plates or on PEI-Cellulose anionic-exchange plates (Merck, Darmstadt). Column chromatography was carried out with a peristaltic pump on silica gel 60 and on Fractogel TSK DEAE 650 (s) ion-exchange resin. Preparative reversed-phase HPLC was carried out on a SuperPac (4 x 250 mm, C_2/C_{18}, 5 µm) Pep-S cartridge (Pharmacia LKB, Sweden) using a Pharmacia LKB HPLC pump and controller (model 2150 and 2152) connected with a variable-wavelength monitor (Knauer, Germany). The analytical HPLC work was performed with a Hewlett-Packard 1090 Series II Liquid Chromatograph with a diode-array detector module (Waldbronn, Germany). Samples were analyzed on a Vydac column (4.6 x 250 mm, C_{18}, 5 µm, The Separations Group, USA) or on a PRP-1 column (4.6 x 120 mm, 5 µm, Hamilton, USA). NMR-spectra were measured on a JNM-GX 400 FT spectrometer (Joel, Japan). Chemical shifts are in ppm relative to $CHCl_3$ as internal standard (^1H) or to external 85% H_3PO_4 (^{31}P) and are positive when downfield from the referencce. Aqueous samples in D_2O were measured in 1 mM EDTA adjusted to pH 5.5. UV spectra were recorded with a Uvicon 810 P spectrometer (Kontron, Germany). Irradiation was performed with a handlamp (Schettler, Germany). T7 DNA polymerase and dNTPs were products from (Boehringer Mannheim, Germany). [α-^{32}P]dCTP (400 Ci/mmol) was purchased from (Amersham, Germany). X-ray films were obtained from Kodak (Great Britain). Ion-spray mass spectrometry (ISMS) analyses were carried out by direct injection on a triplequadrupole Sciex API-III spectrometer (Ontario, Canada).

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Method A

5'-O-(4,4'-dimethoxytrityl)-2'-deoxyadenosine (2)

The O-5' protected nucleoside derivative **2** was prepared according to [26] as follows: 2'-deoxyadenosine (**1**) (5 g, 20 mmol) was dissolved in 50 ml anhydrous pyridine and triethylamine (4.2 ml, 30 mmol), 4-dimethylaminopyridine (60 mg, 0.5 mmol) and 4,4'-dimethoxytrityl chloride (10 g, 30 mmol) in 50 ml pyridine was added. After 4 h the mixture was poured into a 5% cold $NaHCO_3$ solution (100 ml) and extracted (2 x) with 300 ml ethyl acetate. The organic layer was purified on a silica gel column (5 x 60 cm) by eluting first with 3.0 l of $CHCl_3$/MeOH (50:1), and then with 1.5 l of $CHCl_3$/MeOH, (10:1). Yield: 48%. TLC (CH_2Cl_2/MeOH, 19:1): R_f 0.26. UV (MeOH): λ_{max} 259 nm;

λ_{min} 225 nm. ^{1}H-NMR ($CDCl_3$): 8.25 (s, H-C(H8)); 7.97 (s, H-C(H2)); 7.5-7.0 (m, 9 arom. H); 6.9-6.6 (m, 4 arom. H); 6.45 (t, H-C(1',)); 6.16 (br s, NH_2); 4.67 (m, H-C(3')); 4.28 (m, H-C(4')); 3.74 (s, 6 arom. OMe); 3.95-3.90 (m, H-C(5',5")); 3.1-2.3 (m, H-C(2',2")).

5'-O-(4,4'-dimethoxytrityl)-3'-O-methoxyacetyl-2'-deoxyadenosine (3)

Compound **2** (3 g, 5.4 mmol) in anhydrous pyridine (20 ml) was stirred with previously prepared methoxyacetic anhydride (1.05 ml, 6:2 mmol) [17] at room temperature. After 70 min the reaction mixture was evaporated to dryness in vacuo and dissolved in $CHCl_3$/MeOH (100:1) and chromatographed on a column of silica gel (3.5 x 40 cm) with 1.8 l of $CHCl_3$/MeOH (100:1) and then with 0.9 l of $CHCl_3$/MeOH (10:1). Pure **3** (2.0 g, 3.2 mmol) was isolated in 60% yield. TLC ($CHCl_3$/MeOH, 10:1): R_f 0.66. UV (MeOH): λ_{max} 259 nm; λ_{min} 224 nm. ^{1}H-NMR ($CDCl_3$): 8.29 (s, H-C(H8)); 7.98 (s, H-C(H2)); 7.5-7.1 (m, 9 arom. H); 6.9-6.7 (m, 4 arom. H); 6.45 (t, H-C(1',)); 5.87 (br s, NH_2); 5.61 (m, H-C(3')); 4.28 (m, H-C(4')); 4.07 (s, CO-CH_2-O); 3.77 (s, 6 arom. OMe); 3.47 (m, OMe); 3.5-3.4 (m, H-C(5',5")); 3.1-2.5 (m, H-C(2',2")).

3'-O-methoxyacetyl-2'-deoxyadenosine (4)

A solution of the fully protected derivative **3** (2 g, 3.2 mmol) in 80% acetic acid (40 ml) was stirred at room temperature for 3 h. The pale orange solution was evaporated to dryness and the residue dissolved in chloroform (100 ml) and washed with a cold 5% sodium bicarbonate solution (40 ml). The water layer was extracted three times with $CHCl_3$ (20 ml) and the combined organic layers dried with Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified on a silica gel column (2.6 x 40 cm) by elution with $CHCl_3$/MeOH (10:1, 500 ml). The yield of colorless solid was 71% (600 mg, 1.85 mmol). TLC ($CHCl_3$/MeOH, 10:1): R_f 0.66. UV (MeOH): λ_{max} 259 nm; λ_{min} 226 nm. ^{1}H-NMR (CD_3OD): 8.30 (s, H-C(H8)); 8.26 (s, H-C(H2)); 6.41 (t, H-C(1',)); 5.87 (br s, NH_2); 5.55 (m, H-C(3')); 4.24 (m, H-C(4')); 4.14 (s, CO-CH_2-O); 3.77 (s, 6 arom. OMe); 3.86-3.82 (m, H-C(5',5")); 3.44 (m, OMe); 3.1-2.4 (m, H-C(2',2")).

8-Bromo-3'-O-methoxyacetyl-2'-deoxyadenosine (5)

To a 1:1 solution of dioxane and 10% Na_3PO_4 3'-O-methoxyacetyl-2'-deoxyadenosine **4** (220 mg, 0.7 mmol) was added followed by 55 µl of bromine (1 mmol). The mixture was allowed to stir at room temperature in the dark for 5 hours. Then excess bromine was removed by trace amounts of sodium pyrosulfite and extracted three times with $CHCl_3$ (20 ml) and evaporated to dryness. The residue was applied to a silica gel column as above, and eluted with $CHCl_3$/MeOH (7:1, 500 ml), with a yield of 90% (245 mg, 0.6 mmol).

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TLC ($CHCl_3$/MeOH, 7:1): R_f 0.53. UV (MeOH): λ_{max} 264 nm; λ_{min} 230 nm. ^{1}H-NMR ($CDCL_3$): 8.29 (s, H-C(H2)); 6.56 (m, HO-C(5')); 6.40 (t, H-C(1',)); 5.79 (br s, NH_2); 5.68 (m, H-C(3')); 4.28 (m, H-C(4')); 4.13 (s, CO-CH_2-O); 4.00-3.90 (m, H-C(5',5")); 3.50 (s, OMe); 3.3-2.3 (m, H-C(2',2")).

8-Bromo-2'-deoxyadenosine-(1-thiotriphosphate) (7)

The protected 2'-deoxyadenosine derivative **5** (120 mg, 0.3 mmol) was dried [19] and dissolved in anhydrous pyridine (0.3 ml) and dioxane (0.9 ml). During all the following manipulations a weak positive pressure of argon was maintained in the septum-closed reaction vessel by connecting it with an argon-filled tank. All reactions were carried at room temperature. A freshly prepared 1 M solution of salicyl phosphorochloridite (**6**) in anhydrous dioxane (0.66 ml, 0.6 mmol) was added under stirring. After 10 min a well-vortexed mixture of a 0.5 M solution of bis(tri-*n*-butylammonium) pyrophosphate (1.0 ml, 0.5 mmol) anhydrous DMF and tri-*n*-butylamine (0.3 ml) was quickly injected. 10 min later a suspension of sulfur (0.75 ml, 0.75 mmol) in DMF was added followed by an additional 0.3 ml of DMF. After stirring for 10 min, water (5 ml) was added and the reaction mixture was allowed to stir for another 30 min and then evaporated. Removal of the O-5' methoxyacetyl protecting group was carried out in a 1:1 mixture of water and concentrated ammonia (20 ml) at 45° C for 2 ½ h. The reaction mixture was then evaporated to dryness, the residue dissolved in water (10 ml), and applied to DEAE ion-exchange column (2.6 x 20 cm). Chromatography was performed with a linear gradient of 0.7 l each of 0.05 and 0.6 M TEAB (pH 7.7). Fractions of 9 ml were collected, lyophilized, redissolved in MeOH and lyophilized again to give the diastereomeric compound 7 as a white solid (91 mg, 36%). TLC (0.4 M TEAB, pH 7.7) R_f 0.13. UV (10 mM Tris, 7.5): λ_{max} 265 nm; λ_{min} 232 nm. ^{31}P-NMR (H_3PO_4): 43.88-43.62 (2d, P_α), -23.34 to -23,04 (m, P_β), -8.2 to -7.8 (d, P_γ).

Method B

8-Bromo-2'-deoxyadenosine (8)

2'-Deoxyadenosine **1** (780 mg, 2.9 mmol) was dissolved in 90 ml of sodium acetate (1 M; pH 4.0) followed by the addition of bromine (0.22 ml, 4.2 mmol). The reaction mixture was stirred for 3 h in the dark and the work-up was performed as described for compound **5** with a yield of 80%. TLC ($CHCl_3$/MeOH, 7:1): R_f 0.43. UV (MeOH): λ_{max} 265 nm; λ_{min} 232 nm. ^{1}H-NMR (DMSO-d^6): 8.15 (s, H-C(H2)); 7.37 (s, NH_2); 6.36 (t, H-C(H1')); 5.35 (m, HO-C(C5',C2')); 4.43 (s, H-C(H3')); 3.90 (s, H-C(H4')); 3.89-3.50 (m, H-C(H5',H5")); 2.76-2.26 (m, H-C(H2',H2")). ^{13}C-NMR (DMSO-d^6): 155.1 (C6); 152.4 (C2); 149.9 (C4); 126.7 (C8); 119.7 (C5); 88.4 (C1'); 86.4 (C4'); 71.2 (C3'); 62.2 (C5'); 37.1 (C2').

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8-Azido-2'-deoxyadenosine (9)

A solution of previously dried **8** (750 mg, 2.3 mmol) in 20 ml anhydrous DMF was heated with sodium azide (445 mg, 6.8 mmol) and silver sulphate (355 mg, 1.14 mmol) in the dark at 65° C for 14 h. The mixture was filtered and the yellow solution was stored over night at 4° C. A fine white precipitate formed and the solution was then filtered over celite and evaporated. The residue was resuspended twice with 8 ml of EtOH and the solvent was removed in vacuo. 10 ml of cold EtOH was added to the precipitate and filtered, which removed unreacted **8**. This process was repeated a second time with 5 ml of ethanol and the residue was evaporated to dryness, to give a yield of 47%. TLC ($CHCl_3$/MeOH, 7:1): R_f 0.43; PEI-Cellulose (0.4 M, TEAB, pH 7.9). UV (MeOH): λ_{max} 281 nm; λ_{min} 249 nm. ^{1}H-NMR (DMSO-d^6): 8.05 (s, H-C(H2)); 7.27 (s, NH_2); 6.0 (t, H-C(H1')); 5.30 (m, HO-C(C5',C2')); 4.41 (s, H-C(H3')); 3.82 (s, H-C(H4')); 3.64-3.43 (m, H-C(H5',H5")); 3.04-2.08 (m, H-C(H2',H2")). ^{13}C-NMR (DMSO-d^6): 154.4 (C6); 151.55 (C2); 149.3 (C4); 144.1 (C8); 117.1 (C5); 88.1 (C1'); 83.5 (C4'); 71.1 (C3'); 62.1 (C5'); 37.2 (C2').

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8-Azido-2'-deoxyadenosine-5'-O-(1-thiotriphosphate) (11)

The 8-substituted nucleoside **9** was dried further in a desiccator over P_2O_5 under vacuum at room temperature for 24 h. Under a constant flow of Argon 550 mg (1.9 mmol) of **9** was added to 4.5 ml of anhydrous triethyl phosphate and the suspension heated for 2 min at 160° C to dissolve the non-protected nucleoside. The brown, well-vortexed mixture was immediately cooled in ice water. Distilled 2,6-lutidine (0.76 ml, 6.5 mmol) was then injected through a septum followed by $PSCl_3$ (0.35 ml, 3.3 mmol). After stirring in an ice bath for 1 h 40 min, excess $PSCl_3$ was removed in vacuo (4 min). A freshly prepared solution of bis(tri-*n*-butylammonium) pyrophosphate (4.2 g, 9.3 mmol) in 10 ml anhydrous DMF was then injected into the reaction flask and vacuum applied again. After stirring for 4 min the septum was removed and the reaction was stopped by the addition of 10 ml of 2 M TEAB pH 7.9. The precipitate dissolved after vortexing for additional 10 min. The dark redish solution was then lyophilized over night to dryness. The mixture was dissolved in 10 ml 50 mM TEAB pH 7.9 and the non-dissolvable precipitate separated by centrifugation. The solution was chromatographed on a DEAE ion-exchange column as described above and analyzed by TLC (2-propanol/NH_4OH/H_2O, 11:7:2). Fractions containing product were combined and further purified by reversed-phase HPLC. Elution ocurred with 0.1 M TEAB (pH 7.7) containing a linear gradient from 0% to 15% acetonitrile in 20 min and a flow rate of 1 ml per min. Yield: 5%. HPTLC (2-propanol/NH_4OH/H_2O, 11:7:2): R_f 0.19; PEI-Cellulose (0.6 M TEAB, pH 7.9): R_f 0.25. UV (10 mM Tris, 7.5): λ_{max} 265 nm; λ_{min} 232 nm. ^{31}P-NMR (H_3PO_4): 44.34-44.05 (2d, P_α), -23.24 to -23,55 (2d, P_β), -9.50 to -9.63 (d, P_γ).

Incorporation of 8-BrdATPαS and 8-N_3dATPαS into DNA

Incorporation of the newly synthesized compounds 7 and **11** into single-stranded DNA by T7 DNA polymerase were performed in 20-µl samples in a buffer consisting of 40 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 50 mM NaCl and 5 mM DTT. Each sodium salt of compound 7 and **11** (3.4 nmol) was added separately to primer annealed ssmWH14 (8 fmol) obtained from the lab stock, mixed with dGTP, dTTP, dCTP (0.168 nmol) and [α -^{32}P]dCTP (1.5 pmol) and incubated for 1 h at 37° C. In two control experiments dATP (0.168 nmol) and dATPαS (0.168 nmol) were substituted for the derivative. The radioactive samples were applied to a 1% agarose gel. After electrophoresis the gel was exposed to a X-ray film for 2 days at -70° C.

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