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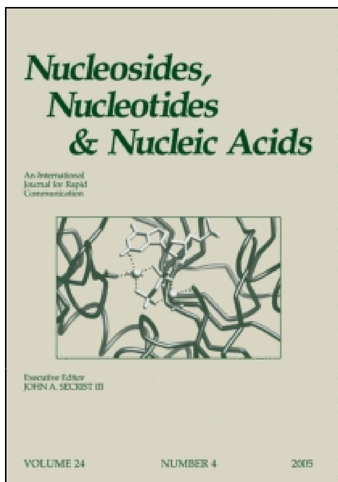

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Synthesis of 2'-Iodo- and 2'-Bromo-ATP and GTP Analogues as Potential Phasing Tools for X-ray Crystallography

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SYNTHESIS OF 2'-IODO- AND 2'-BROMO-ATP AND GTP ANALOGUES AS POTENTIAL PHASING TOOLS FOR X-RAY CRYSTALLOGRAPHY

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ABSTRACT: *Ara*-adenosine (adenine 9-β-D-arabinofuranoside) and *ara*-guanosine (guanine 9-β-D-arabinofuranoside) are converted into 2' halogenated ATP and GTP analogues by triflation and subsequent inversion of configuration at C-2'. For the commercially unavailable *ara*-guanosine a short synthesis starting from guanosine is presented. The nucleotide analogues could serve for the preparation of heavy atom derivatives of ATP- and GTP-binding proteins useful for protein crystal structure determination by MIR/MAD phasing.

INTRODUCTION

X-ray crystallography is the most important method for determination of high resolution protein structures however, the complexity and size of biomacromolecules requires additional estimation of the phases. Currently, the only established crystallographic procedures allowing solution of this phase problem are multiple isomorphous replacement (MIR) and multiwavelength anomalous dispersion (MAD). Both methods take advantage of heavy atoms within the protein crystal that modify observed structure factors. Since only a very few heavy atoms occur naturally in proteins, they have to be

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artificially incorporated. This is usually achieved by modification of either the protein itself or of a protein binding ligand (1).

Modification of the protein is currently the most prominent approach. On the other hand, some ligands such as the nucleotides ATP and GTP are bound with high affinity by a great variety of physiologically important enzymes. Hence, modification of these ligands bears the potential for a universal labeling strategy: The naturally occuring protein-nucleotide affinity could be used for a rational incorporation of heavy atoms into protein crystals. However, this requires that the modification of the nucleotide does not interfere significantly with its protein-binding properties.

Interestingly, for numerous G-proteins a tolerance towards modification of the sugar moiety of GTP has been reported (2). Furthermore, it has been demonstrated that substitution at the 2' position of ATP has only little effect on its affinity to various ATP-binding enzymes. In fact, protein crystals containing the ATP analogue 2'-deoxy-2'-iodoadenosine 5'-triphosphate (2'I-ATP) suitable for X-ray diffraction were obtained (3).

Iodine can be used as heavy atom for MIR, and its chemical analogue bromine is an anomalous scatterer suitable for MAD-phasing. Thus, an efficient synthesis of 2'I- and 2'Br-ATP and -GTP would make them interesting tools for protein structure determination.

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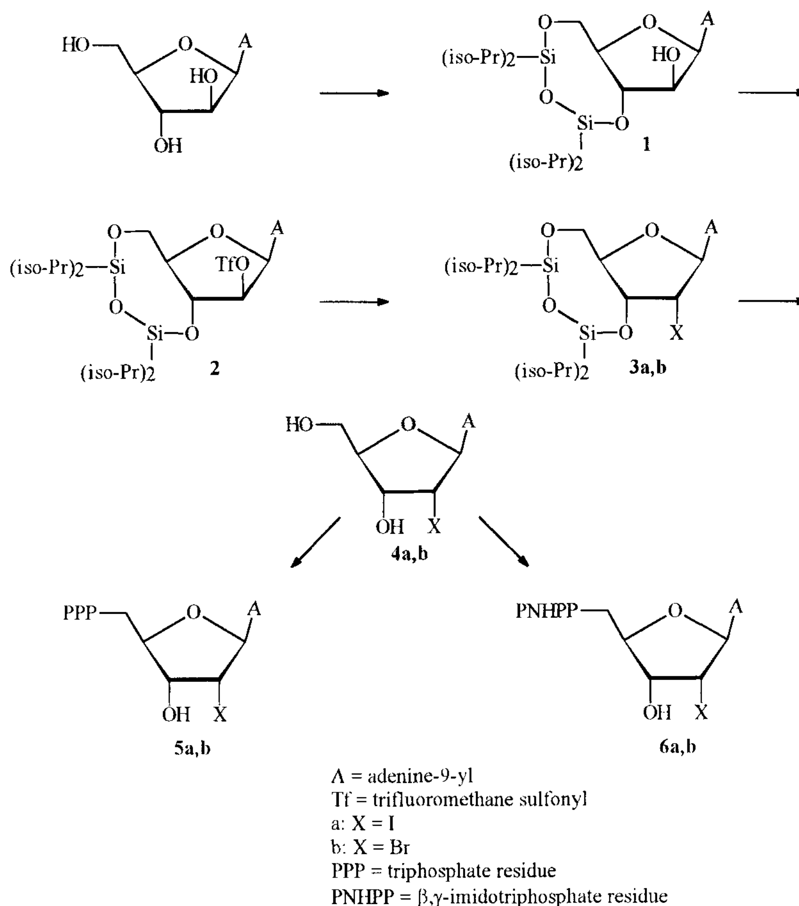
RESULTS

ATP-analogues

The synthesis of 2'-substituted ATP analogues was performed according to the approach of Fukukawa *et al.* (4) using the commercially available *ara*-adenosine as starting material. Simultaneous protection of the 3' and 5' hydroxy functions was achieved with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (TIPDS-Cl_2). Triflation of the 2' hydroxy group and subsequent S_N2-displacement by halogen yielded the 2' deoxy compounds. The TIPDS group was removed with tetra-*n*-butylammonium fluoride and the resulting nucleosides were phosphorylated (FIG 1).

The use of imidodiphosphate instead of pyrophosphate yields triphosphate analogues with an imido function between the β- and the γ-phosphorus of the triphosphate group.



A = adenine-9-yl
Tf = trifluoromethane sulfonyl
a: X = I
b: X = Br
PPP = triphosphate residue
PNHPP = β,γ-imidotriphosphate residue

FIGURE 1

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Although these β,γ-imidotriphosphates show biochemical properties similiar to the triphosphates, the P-N bond cannot be cleaved by most ATP requiring enzymes (5). This stability towards hydrolysis makes β,γ-imidotriphosphates extremely useful in crystallography.

The structures of compounds **5** and **6** were proven by mass spectrometry and NMR. For further verification, analytical samples were treated with alkaline phosphatase or phosphodiesterase. Nucleotide triphosphates are normally stepwise dephosphorylated by alkaline phosphatase whereas β,γ-imidotriphosphates can only be dephosphorylated by the enzyme phosphodiesterase. This enzymatic cleavage can be easily monitored by

HPLC. Treatment of compounds **5a** and **5b** with alkaline phosphatase led via the di- and the monophosphates to the formation of **4a** and **4b**. As expected, compounds **6a** and **6b** were inert to alkaline phosphatase, whereas incubation with phosphodiesterase yielded their monophosphates.

GTP-analogues

The synthesis of 2'-substituted GTP analogues is more complex due to the fact that *ara*-guanosine is commercially unavailable. To date, the only reported synthesis of 2'-deoxy-2'-halogenoguanosines is an eleven step procedure starting from 8-bromoguanosine via N^2-isobutyryl-3',5'-*O*-bis(tetrahydrofuranyl)-D-arabinofuranosylguanine as an intermediate (6-8). Thus, an efficient synthesis of an *ara*-guanosine derivative is the key step for the preparation of the desired nucleotides. A plethora of chemical approaches for the preparation of *ara*-guanosine has been described (9-17) however, these are either expensive or labour-intensive multi step procedures with low overall yields.

For a short and simple synthesis of *ara*-guanosine on a small scale, again the strategy of OH-2' triflation and subsequent S_N2 displacement was used: Guanosine was 3',5' TIPDS protected and the 2' hydroxy function was triflated. Nucleophilic displacement with acetate and subsequent hydrolysis yielded the desired 3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*ara*-guanosine **10.** The TIPDS group was removed with tetra-*n*-butylammonium fluoride and *ara*-guanosine **11** was obtained (FIG 2). The inversion of configuration at C-2' is clearly documented by a 0.4 ppm high field shift of H-2' as well as by a significant change of the 1',2' coupling constant in the ^{1}H-NMR spectra of guanosine and *ara*-guanosine.

With the availability of the *ara*-guanosine derivative **10**, the introduction of halogen can be carried out as for the ATP-analogues. However, the 2' hydroxy function of **10** appeared to be less reactive for triflation than OH-2' of the respective *ribo*-compound. This problem was overcome by the use of the stronger base sodium hydride instead of triethylamine. Subsequent S_N2 displacement with iodide or bromide and removal of the TIPDS group yielded the 2'-deoxy compounds **14a** and **14b**.

Although most G-proteins hydrolyze GTP very slowly (18), GTP cannot normally be used directly for crystallographic purposes. Thus, instead of GTP, the non hydrolyzable GPPNHP is commonly used for the characterisation of the GTP-state of G-proteins (19-

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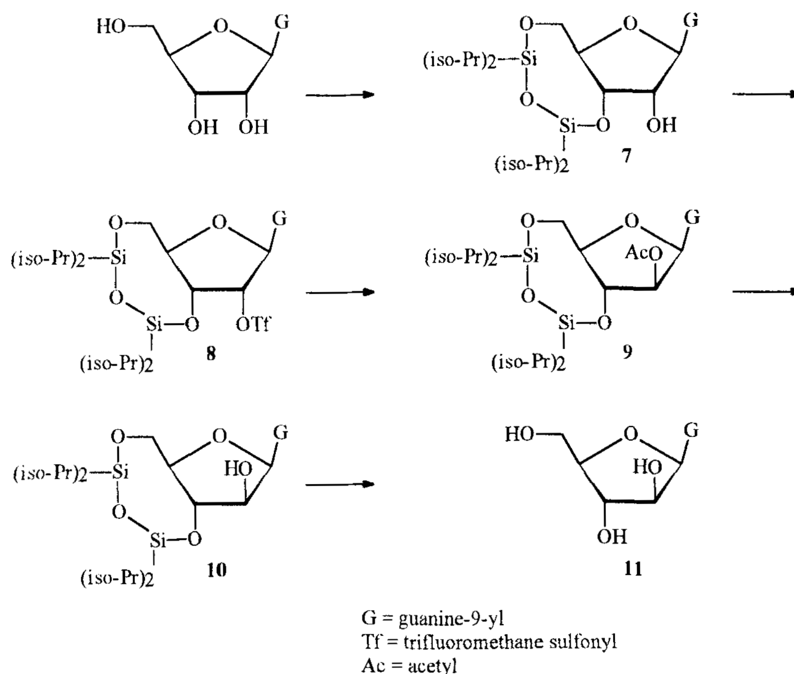

FIGURE 2

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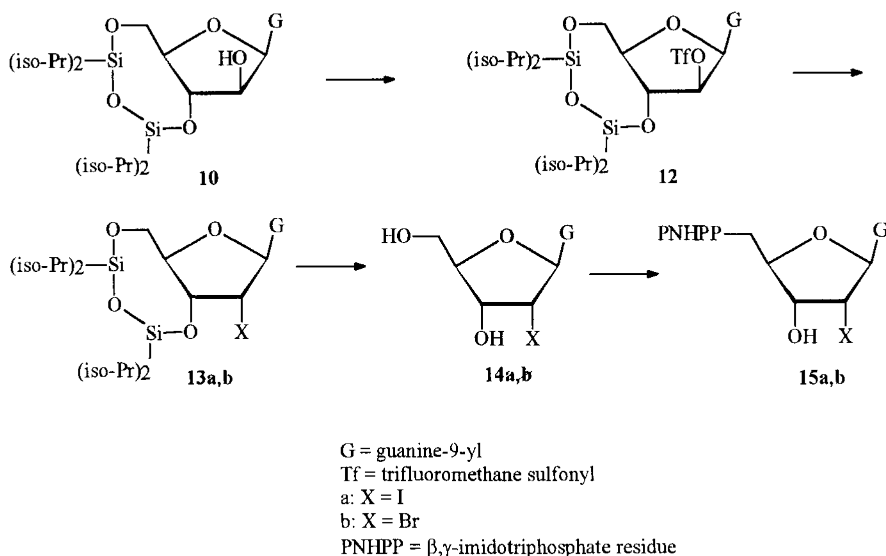
22). For this reason, the compounds **14a** and **14b** were only converted into their imidotriphosphates **15a** and **15b**, respectively (FIG 3). The structures were proven by mass spectrometry and NMR. **15a** and **15b** were inert to alkaline phosphatase whereas incubation with phosphodiesterase yielded the monophosphates.

DISCUSSION

Nucleotide binding proteins play a major role in metabolic processes. Energy requiring biochemical reactions use the enzymatic hydrolysis of ATP, whereas GTP/GDP binding of small G-proteins is a key step for cellular signal transduction. Mutations within these proteins result in a variety of disorders such as cardiac disease or cancer (23,24). Thus, ATP- and GTP-binding proteins are important targets for structure determination and drug development.

The described nucleotide analogues can contribute to an improved structure determination of those proteins. The substitution at C-2' of the nucleotide is likely to be



G = guanine-9-yl
Tf = trifluoromethane sulfonyl
a: X = I
b: X = Br
PNHPP = β,γ-imidotriphosphate residue

FIGURE 3

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tolerated by a large number of proteins and the analogues can be used for a rational incorporation of iodine or bromine into the protein-nucleotide complex. Iodine is widely used as heavy atom for MIR (25) whereas bromine is especially suitable for MAD-phasing (26). With the presented syntheses, the nucleotide analogues are readily available which makes them potential phasing tools for a variety of ATP- and GTP-binding enzymes.

In addition, the compound 3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-*ara*-guanosine **10** was obtained as an intermediate in the synthesis of the GTP analogues. This compound was converted into *ara*-guanosine, which itself is of great pharmaceutical interest (27). Using guanosine as the starting material, this four-step synthesis of an *ara*-guanosine derivative is the most simple and non expensive on a small laboratory scale.

EXPERIMENTAL

General Procedures

NMR spectra were recorded on BRUKER AVANCE DRX 500 spectrometer. Spectra of nucleosides were measured in $[D_6]$DMSO using internal DMSO (2.49 ppm) for

calibration. Spectra of nucleotide triphosphates were recorded in D_2O using internal H_2O (4.81 ppm) or 85% phosphoric acid (0 ppm) as references for ^{1}H- and ^{31}P-NMR, respectively. Mass spectra were recorded on FINNIGAN MAT 95 or FINNIGAN LCQ spectrometer using electrospray ionisation in the negative or positive ion mode. Analytical and preparative HPLC were accomplished with the BECKMAN SYSTEM GOLD on BISCHOFF ODS Hypersil reversed phase C-18 columns (250 x 4.6 or 250 x 8 mm) with one of two buffers: system 1: triethylammoniumacetate pH 6.5 100mM/acetonitrile; system 2: KPi pH 6.5 100 mM with tetrabutylammonium bromide 10 mM/acetonitrile. Ion exchange chromatography was performed on PHARMACIA LKB on a Q-sepharose fast flow column using a linear triethylammonium bicarbonate gradient of 0.05-0.5 M. Enzymatic dephosphorylation was performed at approximately 1U enzyme/100 nmol nucleotide at pH 7.5 at room temperature for 5-100 min using HPLC (system 2) for analysis. Analytical thin layer chromatography was carried out on FLUKA silica gel 60 F_{254} (0.2 mm) or MACHEREY-NAGEL Nano-SIL RP C 18 (0.2 mm) plates. Column flash chromatography was accomplished using MERCK silica gel 60 (230-400 mesh). All reactions were performed under argon atmosphere except where noted otherwise. All reagents were of analytical grade or the best grade available from commercial suppliers.

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Adenosine nucleosides

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-ara-adenosine 1

Adenine 9-β-D-arabinofuranoside (*ara*-adenosine) (435 mg, 1.63 mmol) and imidazole (425 mg, 6.24 mmol) were dissolved in dimethylformamide (5 ml). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (550 μl, 1.73 mmol) was added and the mixture was stirred at room temperature. The progress of the reaction was monitored by TLC. After 50 min the reaction was quenched with ice water (5 ml) and the product was extracted with dichloromethane. The combined organic layers were evaporated and the residue was purified by silica gel flash chromatography. Yield: 789 mg, 1.55 mmol, 95%; r_f: 0.29 dichloromethane/methanol 9/1; ^{1}H-NMR: 1.05 (m, 28H, iso-Pr x 4); 3.80 (m, 1H, H-4'); 4.02 (m, 2H, H-5'a,b); 4.52 (m, 1H, H-2'); 4.58 (dd = t, 1H, H-3'); 5.78 (d, 1H, OH-2'); 6.20 (d, $J_{1',2'}$ = 6.7 Hz, 1H, H-1'); 7.25 (s, 2H, NH_2-6); 8.03, 8.11 (2s, 2H, H-2, H-8)

2'-O-Triflyl-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-ara-adenosine 2

A solution of compound **1** (788 mg, 1.55 mmol), triethylamine (228 µl, 1.64 mmol) and 4-dimethylaminopyridine (198 mg, 1.63 mmol) in dichloromethane (15 ml) was cooled to 0 °C. Trifluoromethane sulfonylchloride (245 µl, 2.33 mmol) was added and the mixture was stirred at room temperature. After 50 min the reaction was quenched with ice water (10 ml) and the product was extracted with dichloromethane. The combined organic layers were evaporated and the residue was purified by silica gel flash chromatography. Yield: 700 mg, 1.09 mmol, 70%; r_f: 0.66 dichloromethane/methanol 9/1, r_f (RP C-18): 0.25 acetonitrile/water 9/1; ^{1}H-NMR: 1.15 (m, 28H, iso-Pr x 4); 3.95 (m, 1H, H-4'); 4.03 (m, 2H, H-5'a,b); 4.67 (dd, 1H, H-3'); 4.59 (dd = t, 1H, H-2'); 6.50 (d, $J_{1',2'}$ = 7.1 Hz, 1H, H-1'); 7.27 (s, 2H, NH_2-6); 8.03, 8.12 (2s, 2H, H-2, H-8)

2'-Deoxy-2'-iodo-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-adenosine 3a

Compound **2** (338 mg, 0.53 mmol) was dissolved in hexamethylphosphoric triamide (6 ml) and lithium iodide (308 mg, 2.3 mmol) was added. The mixture was allowed to stir at room temperature and the progress of the reaction was monitored with TLC. After 24 h the reaction was quenched with ice water (5 ml) and the product was extracted with dichloromethane. The solvent was removed under vacuum at 80 °C and the dry residue purified by silica gel flash chromatography. Yield: 260 mg, 0.42 mmol, 79%; r_f: 0.66 dichloromethane/methanol 9/1, r_f (RP C-18): 0.10 acetonitrile/water 9/1; ^{1}H-NMR: 1.03 (m, 28H, iso-Pr x 4); 3.99 (m, 3H, H-4', H-5'a,b); 4.39 (dd, 1H, H-3'); 5.26 (dd, 1H, H-2'); 6.50 (d, $J_{1',2'}$ = 3.7 Hz, 1H, H-1'); 7.36 (s, 2H, NH_2-6); 8.09, 8.25 (2s, 2H, H-2, H-8)

2'-Deoxy-2'-bromo-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-adenosine 3b

Compound **2** (360 mg, 0.56 mmol) was dissolved in hexamethylphosphoric triamide (6 ml) and lithium bromide (486 mg, 5.60 mmol) was added. The mixture was allowed to stir at room temperature and the progress of the reaction was monitored with TLC. After 3 h the reaction was quenched with ice water (5 ml) and the product was worked up and purified as described for compound **3a**. Yield: 273 mg, 0.48 mmol, 85%; r_f: 0.66 dichloromethane/methanol 9/1, r_f (RP C-18): 0.10, acetonitrile/water 9/1; ^{1}H-NMR: 1.03

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(m, 28H, iso-Pr x 4); 4.04 (m, 3H, H-4', H-5'a,b); 4.99 (dd, 1H, H-3'); 5.28 (dd, 1H, H-2'); 6.43 (d, $J_{1',2'}$ = 2.1 Hz, 1H, H-1'); 7.37 (s, 2H, NH_2-6); 8.08, 8.24 (2s, 2H, H-2, H-8)

2'-Deoxy-2'-iodoadenosine ***4a***

A mixture of compound **3a** (258 mg, 0.42 mmol), tetra-*n*-butylammonium fluoride (1.0 ml of a 1 M solution in tetrahydrofurane) and tetrahydrofurane (1.5 ml) was stirred at room temperature. After 30 min the solvent was evaporated and the residue was purified by silica gel flash chromatography. Yield: 132 mg, 0.35 mmol, 83%; r_f: 0.23 dichloromethane/methanol 9/1; ^{1}H-NMR: 3.65 (m, 2H, H-5'a,b); 4.08 (m, 2H, H-4' and H-3'); 5.11 (dd, 1H, H-2'); 5.37 (t, 1H, OH-5'); 6.09 (d, 1H, OH-3'); 6.40 (d, $J_{1',2'}$ = 9.0 Hz, 1H, H-1'); 7.37 (s, 2H, NH_2-6); 8.14, 8.37 (2s, 2H, H-2, H-8)

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2'-Deoxy-2'-bromoadenosine ***4b***

Compound **3b** (273 mg, 0.48 mmol), tetra-*n*-butylammonium fluoride (1.1 ml of a 1 M solution in tetrahydrofurane), tetrahydrofurane (1.5 ml). Reaction and work up were performed as described for compound **4a**. Yield: 113 mg, 0.34 mmol, 71%; r_f: 0.35 dichloromethane/methanol 9/1; ^{1}H-NMR: 3.68 (m, 2H, H-5'a,b); 4.07 (m, 1H, H-4'); 4.30 (m, 1H, H-3'); 5.13 (dd, 1H, H-2'); 5.42 (t, 1H, OH-5'); 6.06 (d, 1H, OH-3'); 6.30 (d, $J_{1',2'}$ = 7.7 Hz, 1H, H-1'); 7.37 (s, 2H, NH_2-6); 8.13, 8.40 (2s, 2H, H-2, H-8)

Guanosine nucleosides

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-guanosine 7

A suspension of guanosine (4.0 g, 14.2 mmol) and imidazole (2.4 g, 34.6 mmol) in DMF (40 ml) was stirred at room temperature. TIPDS-Cl_2 (4.5 ml, 14.2 mmol) was added and the progress of the reaction was minitored by TLC. After addition of TIPDS-Cl_2 the mixture became a clear solution and after 45 min the reaction was quenched with ice water (30 ml). The precipitate was filtered, washed with water several times and dried under vacuum. Yield: 7.13 g, 13.5 mmol, 95 %; r_f: 0.42 dichloromethane/methanol 9/1;^{1}H-NMR: 1.02 (m, 28H, iso-Pr x 4); 3.94 (dd, 1H, H-5'a); 3.99 (m, 1H, H-4'); 4.08 (dd, 1H, H-5'b); 4.27 (m, 1H, H-2'); 4.36 (dd, 1H, H-3'); 5.60 (d, 1H, OH-2'); 5.68 (d, $J_{1',2'}$ = 1.6 Hz, 1H, H-1'); 6.48 (s, 2H, NH_2-2); 7.77 (s, 1H, H-8); 10.64 (s, 1H, H-1)

2'-O-Triflyl-3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-guanosine ***8***

7 (4.0 g, 7.6 mmol), triethylamine (1.22 ml, 8.76 mmol) , 4-dimethylaminopyridine (1.08 g, 8.84 mmol), trifluoromethane sulfonylchloride (1.20 ml, 11.2 mmol), dichloromethane (50 ml). Reaction and work up were performed as described for compound **2**. Yield: 3.2 g, 4.9 mmol, 64%; r_f: 0.58 dichloromethane/methanol 9/1; ^{1}H-NMR: 1.01 (m, 28H, iso-Pr x 4); 3.98 (m, 2H, H-5'a und H-4'); 4.08 (dd, 1H, H-5'b), 4.75 (dd, 1H, H-3'); 5.92 (dd, 1H, H-2'); 6.12 (d, $J_{1',2'}$ = 1,2 Hz, 1H, H-1'); 6.35 (s, 2H, NH_2-2); 7.80 (s, 1H, H-8); 10.72 (s, 1H, H-1)

2'-O-Acetyl-3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-ara-guanosine ***9***

A suspension of compound **8** (3.0 g, 4.6 mmol) and sodium acetate (3.8 g; 46 mmol) in hexamethylphosphoric triamide (30 ml) was stirred at room temperature. The progress of the reaction was monitored by TLC. After 20 h the reaction was quenched with ice water (20 ml) and the product was extracted with ethyl acetate. The solvent was removed under vacuum at 80 °C and the dry residue purified by silica gel flash chromatography. Yield: 1.2 g, 2.1 mmol, 46%; r_f: 0.56 dichloromethane/methanol 9/1; ^{1}H-NMR: 1.02 (m, 28H, iso-Pr x 4); 1.74 (s, 3H, OAc-2'); 3.95 (m, 2H, H-5'a and H-4'); 4.06 (dd, 1H, H-5'b); 4.55 (dd = t, 1H, H-3'); 5.53 (dd, 1H, H-2'); 6,15 (d, $J_{1',2'}$ = 7,1 Hz, 1H, H-1'); 6.46 (s, 2H, NH_2-2); 7.64 (s, 1H, H-8); 10.60 (s, 1H, H-1)

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3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-ara-guanosine ***10***

A suspension of **9** (1.2 g, 2.1 mmol) in methanol/dioxane/25% aqueous ammonia (25 ml, 1:1:1,5) was stirred at room temperature for 24 h. The solvent was evaporated and the residue was purified by silica gel flash chromatography. Yield: 422 mg, 0.80 mmol, 38 %; r_f: 0.31 dichloromethane/methanol 9/1; ^{1}H-NMR: 1.02 (m, 28H, iso-Pr x 4); 3.73 (m, 1H, H-4'); 3.93 (m, 2H, H-5'a,b); 4.29 (dd = t, 1H, H-3'); 4.40 (m, 1H, H-2'); 5.79 (d, 1H, OH-2'); 5.94 (d, $J_{1',2'}$ = 6.3 Hz, 1H, H-1'); 6.45 (s, 2H, NH_2-2); 7.69 (s, 1H, H-8); 10.60 (s, 1H, H-1)

Ara-guanosine ***11***

A mixture of compound **10** (20 mg, 38 μmol), tetra-n-butylammonium fluoride (80 μl of a 1 M solution in tetrahydrofurane) and tetrahydrofurane (200 μl) was stirred at room

temperature. After 30 min the solvent was evaporated and the residue was purified by HPLC (system 1). Yield: 5 mg, 18 µmol, 47 %; ^{1}H-NMR: 3.58 (m, 2H, H-5'a,b); 3.72 (m, 1H, H-4'); 4.03 (m, 2H, H-3' und H-2'); 5.03 (t, 1H, OH-5'); 5.47 (d, 1H, OH-2'); 5.58 (d, 1H, OH-3'); 5.99 (d, $J_{1',2'}$ = 4.6 Hz, 1H, H-1'); 6.43 (s, 2H, NH_2-2); 7.73 (s, 1H, H-8); 10.54 (s, 1H, H-1)

*2'-O-Triflyl-3',5'-O-(tetraisopropyldisiloxan-1,3-diyl)-ara-guanosine **12***

Compound **10** (400 mg, 0.76 mmol), 4-dimethylaminopyridine (44 mg, 0.34 mmol) and sodium hydride (96 mg, 3.9 mmol) were suspended in THF (14 ml). The mixture was stirred at room temperature and after 1 h it was cooled to 0 °C. Trifluoromethane sulfonylchloride (336 µl, 3.26 mmol) was added and the progress of the reaction was monitored by TLC. After 30 min the reaction was quenched with a saturated sodium bicarbonate solution and the product was extracted with dichloromethane. The combined organic layers were evaporated to dryness and the residue was purified by silica gel flash chromatography. Yield: 235 mg, 0.35 mmol, 46%; r_f: 0.58 dichloromethane/methanol 9/1; ^{1}H-NMR: 1.02 (m, 28H, iso-Pr x 4); 4.01 (m, 3H, H-4' and H-5'a,b); 4.71 (m, 1H, H-3'); 5.98 (dd, 1H, H-2'); 6.29 (d, $J_{1',2'}$ = 6.8 Hz, 1H, H-1'); 6.47 (s, 2H, NH_2-2); 7.82 (s, 1H, H-8); 10.64 (s, 1H, H-1)

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*2'-Deoxy-2'-iodo-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-guanosine **13a***

Compound **12** (97 mg, 0.15 mmol); lithium iodide (203 mg, 1.47 mmol); hexamethylphosphoric triamide (3 ml). Reaction and work up were performed as described for compound **3a**. Yield: 60 mg, 94 µmol, 63%; r_f: 0.46 dichloro-methane/methanol 9/1; ^{1}H-NMR: 1.03 (m, 28H, iso-Pr x 4); 3.95 (dd, 1H, H-5'a); 3.98 (m, 1H, H-4'); 4.05 (dd, 1H, H-5'b); 4.34 (dd, 1H, H-3'); 5.09 (m, 1H, H-2'); 6.14 (d, $J_{1',2'}$ = 6 Hz, 1H, H-1'); 6.50 (s, 2H, NH_2-2); 7.83 (s, 1H, H-8); 10.66 (s, 1H, NH-1)

*2'-Deoxy-2'-bromo-3',5'-O-(tetraisopropyldisiloxane-1,3-diyl)-guanosine **13b***

Compound **12** (135 mg, 0.20 mmol); lithium bromide (179 mg, 2.1 mmol); hexamethylphosphoric triamide (4 ml). Reaction and work up were performed as described for compound **3a**. Yield: 57 mg, 97 µmol, 47%; r_f: 0.53 dichlorome-

thane/methanol 9/1; ^{1}H-NMR: 1.02 (m, 28H, iso-Pr x 4); 3.91 (dd, 1H, H-5'a); 3.97 (m, 1H, H-4'); 4.05 (dd, 1H, H-5'b); 4.47 (dd, 1H, H-3'); 5.10 (m, 1H, H-2'); 6.13 (d, $J_{1',2'}$ = 7.0 Hz, 1H, H-1'); 6.51 (s, 2H, NH_2-2); 7.79 (s, 1H, H-8); 10.65 (s, 1H, NH-1)

*2'-Deoxy-2'-iodoguanosine **14a***

A mixture of compound **13a** (50 mg, 79 µmol); tetra-n-butylammonium fluoride (160 µl of a 1M solution in tetrahydrofurane) and tetrahydrofurane (2.5 ml) was allowed to stir at room temperature for 30 min. After evaporation of the solvent the resulting residue was washed with methanol several times. An analytical sample was purified by HPLC (system 1). Yield: 16 mg, 41 µmol, 52%; ^{1}H-NMR: 3.61 (m, 2H, H-5'a,b); 4.00 (m, 2H, H-4' and H-3'); 4.96 (dd, 1H, H-2'); 5.11 (t, 1H, OH-5'), 5.98 (d, 1H, OH-3'); 6.04 (d, $J_{1',2'}$ = 8.6 Hz, 1H, H-1'); 6.48 (s, NH_2-2); 7.96 (s, 1H, H-8); 10.60 (s, 1H, NH-1); MS m/z: 394 Da $(M+H)^+$

*2'-Deoxy-2'-bromoguanosine **14b***

Compound **13b** (50 mg, 85 µmol) tetra-n-butylammonium fluoride (160 µl of a 1M solution in tetrahydrofurane); tetrahydrofurane (2.5 ml). The reaction was performed as described for compound **14a**. Yield: 12 mg, 35 µmol, 41%; ^{1}H-NMR: 3.61 (m, 2H, H-5'a,b); 3.98 (m, 1H, H-4'); 4.22 (m, 1H, H-3'); 4.97 (dd, 1H, H-2'); 5.13 (t, 1H, OH-5'); 5.97 (d, 1H, OH-3'); 6.02 (d, $J_{1',2'}$ = 7.6 Hz, 1H, H-1'); 6.53 (s, 2H, NH_2-2); 7.69 (s, 1H, H-8); 10.60 (s, 1H, H-1); MS m/z: 346/348 Da $(M+H)^+$

Phosphorylation

Phosphorylation was performed according to the method of Ludwig (28). Briefly, 0.03-0.20 mmol of nucleoside were dissolved in trimethyl phosphate (0.5 ml) and 3-4 equivalents of phosphorus oxychloride were added. The mixture was allowed to stir at 0 °C for 45-90 min and the progress of the reaction was monitored with HPLC (system 2). At the apparent maximum of formation of the phosphorodichloridate intermediate, the excess of phosphorus oxychloride was removed by brief evaporation, and 5 equivalents of bis-tri-*n*-butylammonium pyrophosphate in dimethylformamide (1 ml) and tributylamine (0.5 ml) were added. The reaction was quenched after 1-1.5 min with 0.2

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M aqueous triethylammonium bicarbonate (3 ml). For the syntheses of imidotriphosphates bis-tri-*n*-butylammonium imidodiphosphate was prepared from its sodium salt according to the method of Moffat (29). The mixture was evaporated at 20-25 °C and water (20 ml) was added. The pH was adjusted to 7.5 and the aqueous layer was extracted with ether in order to remove excess of amines. After brief evaporation the product was purified by ion exchange chromatography. The fractions estimated to contain the product were combined and evaporated to dryness at 20-25 °C. Methanol (5 ml) was added and evaporated to dryness several times. The product was dissolved in water and the pH adjusted to 7.5 with NaOH. The product was divided into small fractions, shock frozen in liquid nitrogen and stored at -18 °C.

2'-Deoxy-2'-iodoadenosine 5'-triphosphate (2'I-ATP) ***5a***

Yield: 10 mg, 16 μmol, 12%; ^{1}H-NMR: 4.22 (m, 2H, H-5'a,b); 4.38 (m, 1H, H-4'); 4.44 (m, 1H, H-3'); 4.96 (m, 1H, H-2'); 6.40 (d, 1H, H-1'); 8.19, 8.45 (2s, 2H, H-2, H-8); ^{31}P-NMR: -10.3 (d); -11.4 (d); -23.2 (t); MS m/z: 239 Da (PPP)$^-$, 616 Da (M-H)$^-$

2'-Deoxy-2'-bromoadenosine 5'-triphosphate (2'Br-ATP) ***5b***

Yield: 51 mg, 83 μmol, 49%; ^{1}H-NMR: 4.30 (m, 2H, H-5'a,b); 4.49 (m, 1H, H-4'); 4.68 (m, 1H, H-3'); 5.01 (m, 1H, H-2'); 6.42 (d, 1H, H-1'); 8.24, 8.53 (2s, 2H, H-2, H-8); ^{31}P-NMR: -10.1 (d); -11.4 (d); -23.2 (t); MS m/z: 239 Da (PPP)$^-$, 568/570 Da (M-H)$^-$

2'-Deoxy-2'-iodoadenosine 5'-(β,γ-imido)-triphosphate (2'I-APPNHP) ***6a***

Yield: 25 mg, 40 μmol, 24%; ^{1}H-NMR: 4.28 (m, 2H, H-5'a,b); 4.43 (m, 1H, H-4'); 4.52 (m, 1H, H-3'); 4.99 (m, 1H, H-2'); 6.48 (d, 1H, H-1'); 8.35, 8.57 (2s, 2H, H-2, H-8); ^{31}P-NMR: -10.5 (d); -11.7 (d); -19.5 (t); MS m/z: 238 Da (PPNHP)$^-$, 615 Da (M-H)$^-$

2'-Deoxy-2'-bromoadenosine 5'-(β,γ-imido)-triphosphate (2'Br-APPNHP) ***6b***

Yield: 18 mg, 31 μmol, 20%; ^{1}H-NMR: 4.33 (m, 2H, H-5'a,b); 4.51 (m, 1H, H-4'); 4.66 (m, 1H, H-3'); 4.97 (m, 1H, H-2'); 6.43 (d, 1H, H-1'); 8.33, 8.56 (2s, 2H, H-2, H-8); ^{31}P-NMR: -10.3 (d); -11.8 (d); -19.5 (t); MS m/z: 238 Da (PPNHP)$^-$, 567/569 Da (M-H)$^-$

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2'-Deoxy-2'-iodoguanosine 5'-(β,γ-imido)-triphosphate (2'I-GPPNHP) ***15a***

Yield: 2 mg, 3 μmol, 10%; ^{1}H-NMR: 4.04 (m, 2H, H-5'a,b); 4.31 (m, 1H, H-4'); 4.55 (m, 1H, H-3'); 4.81 (m, 1H, H-2'); 6.24 (d, 1H, H-1'); 8.07 (s, 1H, H-8); ^{31}P-NMR: -10.4 (d); -11.4 (d); -19.6 (t); MS m/z: 238 Da (PPNHP)$^{-}$

2'-Deoxy-2'-bromoguanosine 5'-(β,γ-imido)-triphosphate (2'Br-GPPNHP) ***15b***

Yield: 2 mg, 3 μmol, 13%; ^{1}H-NMR: 4.16 (m, 2H, H-5'a,b); 4.32 (m, 1H, H-4'); 4.58 (m, 1H, H-3'); 4.83 (m, 1H, H-2'); 6.29 (d, 1H, H-1'); 8.30 (s, 1H, H-8); ^{31}P-NMR: -10.5 (d); -11.7 (d); -19.6 (t); MS m/z: 238 Da (PPNHP)$^{-}$, 583/585 Da (M-H)$^{-}$

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