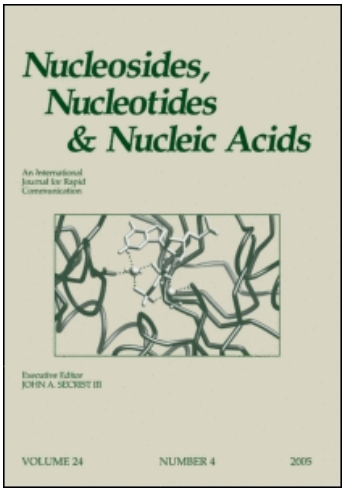


Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

A New Approach to the Synthesis of 2-Aminopurine-2'-deoxyriboside *via* Tri-n-butyltin Hydride Reduction

Sabine Schmidt[a]; Dieter Cech[a]
[a] Institut für Chemie, Humboldt-Universität zu Berlin, Berlin, Germany

To cite this Article Schmidt, Sabine and Cech, Dieter(1995) 'A New Approach to the Synthesis of 2-Aminopurine-2'-deoxyriboside *via* Tri-n-butyltin Hydride Reduction', Nucleosides, Nucleotides and Nucleic Acids, 14: 6, 1445 — 1452
To link to this Article: DOI: 10.1080/15257779508010704
URL: http://dx.doi.org/10.1080/15257779508010704

NUCLEOSIDES & NUCLEOTIDES, 14(6), 1445-1452 (1995)

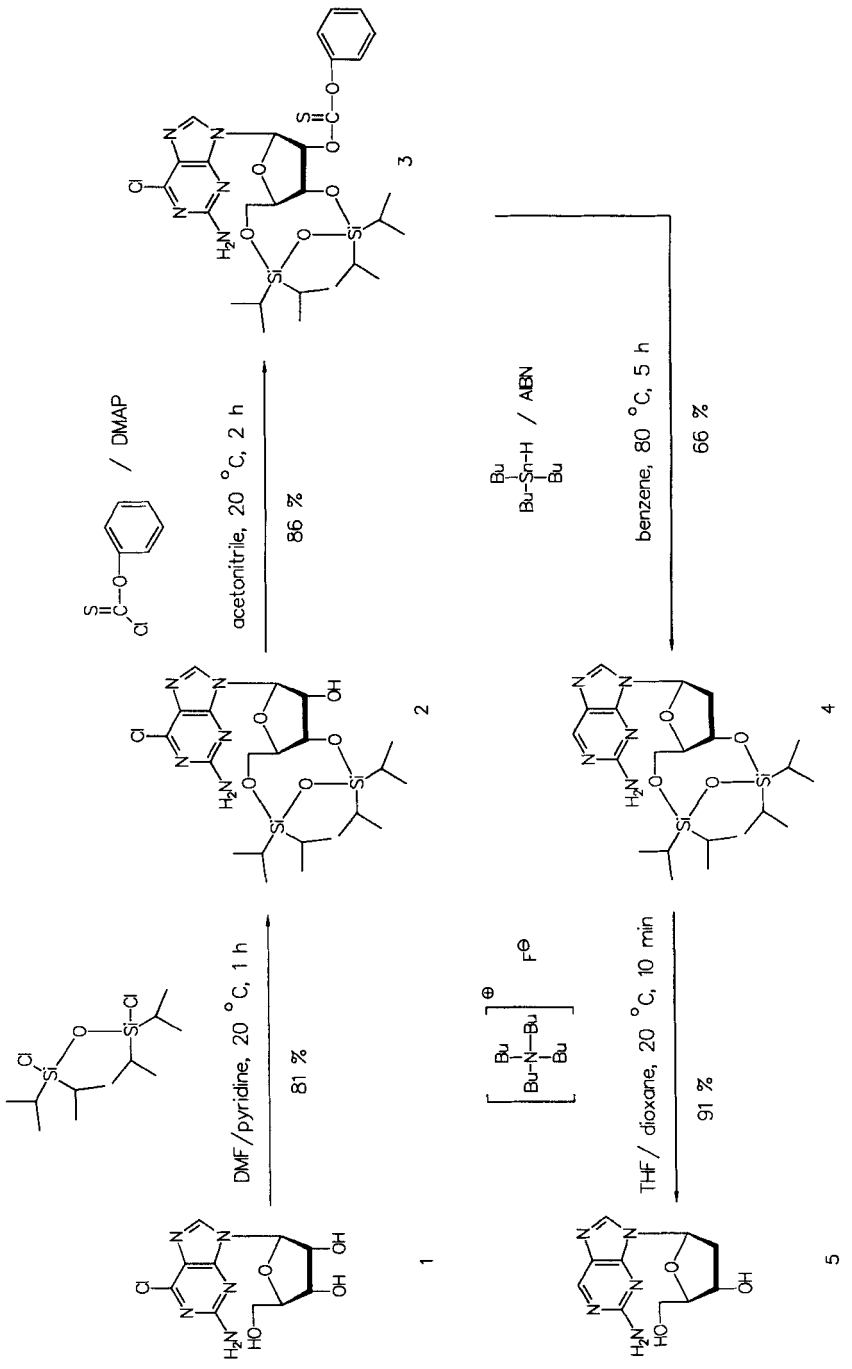
A NEW APPROACH TO THE SYNTHESIS OF 2-AMINOPURINE-2'-DEOXYRIBOSIDE *VIA* TRI-n-BUTYLTIN HYDRIDE REDUCTION

Sabine Schmidt and Dieter Cech*
Institut für Chemie, Humboldt-Universität zu Berlin, Hessische Str. 1-2, 10099 Berlin, Germany

ABSTRACT: A simple conversion of guanosine to 2-aminopurine-2'-deoxyriboside is described. Following the synthesis of the 2'-phenoxythiocarbonyl derivative of 6-chloroguanosine, 2-aminopurine-2'-deoxyriboside can be prepared by simultaneous reduction of both the 6- and 2'-position using tri-n-butyltin hydride as reducing agent. Several oligonucleotides containing 2-aminopurine-2´-deoxyriboside have been synthesized.

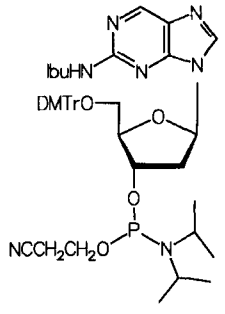
2-Aminopurine is a much studied nucleobase analogue with interesting biological properties. Its mutagenity, the fluorescence properties as well as its structural homology to adenine and guanine make it an important tool in enzymological studies. Therefore, different routes to the synthesis of 2-aminopurine riboside and the corresponding 2'-deoxyriboside **5** have been developed[1-5]. Here we report a new synthetic route to 2-aminopurine-2'-deoxyriboside, which is characterized by mild reaction conditions, reagents of simple handling and high yields.

Previously described chemical syntheses of 2-aminopurine-2'-deoxyriboside make use of 2'-deoxyguanosine as starting material[3,4]. The synthesis of the hydrazino derivative and its treatment with silver oxide to yield 2-aminopurine-2'-deoxyriboside[3] is characterized by low effectivity, the thiation of 2'-deoxyguanosine by NaSH[4] is very time-consuming and the use of molecular hydrogen, as recently reported[5], is also not very convenient for any scale-up procedure. Additionally, the high lability of the glycosidic bond of 2'-deoxyguanosine requires very mild reaction conditions or a partial degradation of the starting material will take place during the multistep synthesis. An alternative is offered by using guanosine instead of 2'-deoxyguanosine as starting material. Simultaneous reduction at both positions 6 and 2' could lead to a very efficient and time saving synthesis of 2-aminopurine-2'-deoxyriboside. Originally, the phenoxythiocarbonyl group was chosen as reducible protecting group for both positions[6]. The 2'-thiocarbonylester



makes the 2'-carbon-oxygen bond amenable to reduction by tri-n-butyltin hydride[7]. In principle, the same reaction should be applicable to the oxygen atom at position 6. Its activation by phenoxythiocarbonyl chloride, however, failed. Therefore, introduction of a different reducible group at C-6 was required. Carbon-halogen-bonds have been employed earlier for homolytic cleavage. Thus, the 6-chloro derivative of guanosine[8] was used as starting compound. After protection of the 3'- and 5'- hydroxyl groups by 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (Markiewicz reagent)[9], the 2'-hydroxyl group of the protected 6-chloro-guanosine **2** was transformed to the phenoxythiocarbonyl ester **3**, which was subjected to reduction by tri-n-butyltin hydride. Both the 6-position of the heterocyclic base and the 2'-position of the sugar moiety were converted simultaneously giving 2-aminopurine-2'-deoxyriboside **5** in satisfactory yield. The intermediate products **3** and **4** as well as the final product **5**, were isolated and purified by silica gel chromatography using chloroform/methanol gradients.

The method described here represents a very efficient synthesis of 2-aminopurine-2'-deoxyriboside that has certain advantages over existing methods. Unlike the reported syntheses of 2-aminopurine derivatives[3,5], the procedure does not require any protection of the exocyclic amino function in the starting material. With the Markiewicz reagent the 3'- and 5'-groups are effectively blocked, the remaining unblocked 2'-hydroxyl function can be carboxylated smoothly by phenoxythiocarbonyl chloride in the next step. Tri-n-butyltin hydride is a mild reducing agent. It can be easily removed at the end of the reaction, whereas the removing of silver oxide in the reported oxidation procedure of 2-amino-6-hydrazinopurine[3] is troublesome. Thus, **5** was obtained in yields of about 60 %. The correct structure of **5** was confirmed both by ^{1}H-NMR spectroscopy and mass spectrometry. The obtained data were in good agreement with the analytical data reported by Jones et al.[4]. In addition, HPLC analysis and UV spectroscopy have confirmed **5** as 2-aminopurine-2'-deoxyriboside by comparison with authentic material.



6

The modified nucleoside is of partial interest for incorporation into oligonucleotides which can be used for enzymological studies. Thus, to study the use of **5** as starting material in automated oligonucleotide synthesis, we have prepared a fully protected phosphoramidite building block of 2-aminopurine-2'-deoxyriboside **6** and synthesized the following modified oligonucleotides:

d(TCTGCGACAGATTCCTΠGGATAAGCCAAGT),
d(GCCAACCTΠGCTCT), d(GCCAACCTGΠCTCT), d(GCCAACCTΠΠCTCT),
d(AGAGCCAΠGTTGGC), d(AGAGCCAGΠTTGGC), d(AGAGCCAΠΠTTGGC)
and d(AGAGCCΠGGTTGGC)
Π = 2-aminopurine

Benzoyl protection of **5** proved to be unfavourable, because this group was only incompletely removed even after treatment with concentrated ammonia at 70 °C overnight. Isobutyryl protection turned out to be satisfactory[10]. This group was removed quantitatively during the ammonia treatment already at 50 °C overnight, commonly used in the deprotection scheme of oligonucleotides after synthesis if benzoyl groups are used with 2'-deoxyadenosine and 2'-deoxycytidine and isobutyryl group with 2'-deoxyguanosine. Dimethoxytritylation of N^4-isobutyryl-2-aminopurine-2'-deoxyriboside was carried out by standard procedures[11,12]. Synthesis of the phosphoramidite building block was performed in analogy to procedures described in the literature[13]. Automated synthesis of oligonucleotides containing 2-aminopurine residues was performed following the standard elongation cycle. The incorporation of the 2-aminopurine phosphoramidite was achieved with yields of about 98 % in the coupling reaction. Synthetic oligonucleotides were subjected to nucleoside composition analysis. Samples were digested with snake venom phosphodiesterase and alkaline phosphatase and the resulting nucleosides were separated and identified by HPLC. 2-Aminopurine-2'-deoxyriboside could be identified in each hydrolysed oligonucleotide confirming the successful incorporation of this modified nucleoside into the oligonucleotides.

The sequences of the synthesized oligonucleotides were proved by Maxam-Gilbert sequencing. Under these conditions 2-AP is modified in the same way as adenine. Therefore, in the gel electrophoresis the fragments ending with 2-AP show the same behaviour as those of adenine.

The results of enzymological studies will be published soon[14].

EXPERIMENTAL

Materials and methods. 6-Chloro-guanosine, azobis(isobutyronitrile) and tetrabutylammonium fluoride (1.1 M solution in THF) were purchased from Sigma-Aldrich, Deisenhofen. 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (Markiewicz reagent), phenoxythiocarbonyl chloride and tri-n-butyltin hydride were obtained from Merck, Darmstadt. Dimethylformamide, acetonitrile, benzene, dioxane and pyridine were purchased as dry solvents from Fluka, Ulm and used without further purification. For thin layer chro-

matography (TLC), glass silica gel plates containing a 254 nm fluorescent indicator (Merck, Darmstadt) were used throughout. Column chromatography was performed using silicagel 60 (particle size 0.036-0.2 mm) from Merck, Darmstadt. Column eluates were monitored by UV absorption at 254 nm and analyzed by TLC. ^{1}H-and ^{31}P-NMR and spectra were recorded with a Bruker AM 300 spectrometer. Mass spectrometry was performed using a Hewlett-Packard-5995A GC/MS couple. UV spectra of modified nucleosides were measured in H_2O (pH 7) using a Shimadzu 160 A UV/VIS spectrometer. HPLC was performed using an LC6A gradient system (Shimadzu) and an ICI/GAT gradient system. Buffers were as follows: **A**: 50% 0.1 M TEAA(triethylammonium acetate)/50 % CH_3CN, pH 7; **B**: 98 % 0.1 M TEAA/2 % CH_3CN, pH 7; **C**: 0.04 M KH_2PO_4, pH 4.2; **D**: 0.04 M Tris·HCl, 0.04 M $MgCl_2$, pH 8.9. Phosphodiesterase from *Crotalus durissus* (0.003 u/µl) and calf intestine alkaline phosphatase (22 u/µl) were obtained from Boehringer Mannheim.

2-Amino-6-chloro-9-[β-D-2-phenoxythiocarbonyl-3,5-O-(tetraisopropyl-disiloxane-1,3-diyl)-ribofuranosyl]-purine (3). 0.3 g (1 mmol) 6-chloro-guanosine **1** was dried by coevaporation with anhydrous pyridine and suspended in 5 ml anhydrous dimethylformamide and 0.7 ml pyridine. Markiewicz reagent (0.3 ml in 1 ml dimethylformamide) was added and the mixture stirred at room temperature for 1 h, at which point TLC ($CHCl_3$/CH_3OH 98:2 v/v) showed complete conversion of the starting material to a faster moving product. The reaction was stopped by adding of 5 ml 1 N $NaHCO_3$. After standing for 10 minutes, the mixture was evaporated to dryness and the residue partitioned between $CHCl_3$ and saturated aqueous $NaHCO_3$. The organic layer was washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The product was purified by column chromatography on silica gel using $CHCl_3$ with a gradient of 0-2 % CH_3OH as eluent. The protected 6-chloro-guanosine **2** was dissolved in 15 ml of anhydrous acetonitrile. 0.25 g (1.5 mmol) 4-dimethylaminopyridine and 0.19 g (1.1 mmol) phenoxythiocarbonyl chloride were added and the solution was stirred at room temperature for 2 h. The mixture was evaporated to dryness *in vacuo*. The residue was dissolved in ethyl acetate and extracted repeatedly with saturated aqueous $NaHCO_3$ and saturated aqueous NaCl. The organic layer was dried over Na_2SO_4 and evaporated *in vacuo*. The product was purified by column chromatography on silica gel using $CHCl_3$ with a gradient of 0-2 % CH_3OH as eluent. Yield: 0.61 g (0.7 mmol, 70 %) of **3**; m.p. 61 °C; ^{1}H-NMR ($CDCl_3$): δ[ppm] 1.0-1.1 (m, 28H, isopropylH), 4.0-4.2 (m, 3H, H-4', H-5'), 5.0 (dd, 1H, J_1=5.3, J_2=8.5, H-3'), 5.3 (br, 2H, 2-NH_2), 6.3 (dd, 1H, J_1=5.0, J_2=5.8, H-2'), 7.2 (d, 1H, J=7.7, H-1'), 7.3-7.5 (m, 5H, benzoylH), 7.9 (s, 1H, H-8); MS: m/z 511.2 (protected ribose$^+$, 4.6 %), 170 (base + H^+, 52.8 %), 137 (phenoxythiocarbonyl$^+$, 18.4 %), 135 (base - Cl + 2H^+, 37.5 %).

2-Amino-9-(2-deoxy-β-D-ribofuranosyl)-purine (5). 0.68 g (1 mmol) **3** was dissolved in 10 ml of freshly distilled anhydrous benzene (dried over sodium) containing 48 mg (0.3 mmol) azobis(isobutyronitrile). The solution was degassed with oxygen-free N_2 for 15 min. The solution was degassed with oxygen-free N_2 for further 10 min after adding of 2.7 ml (13.5 mmol) tri-n-butyltin hydride, and than refluxed for 5 h. The reaction was followed by TLC. Formation of the 2-aminopurine nucleoside is indicated by appearance of a new blue fluorescent product. The solvent was evaporated and the residue was chromatographed on silica gel using $CHCl_3$ with a gradient of 0-5 % CH_3OH as eluent. The tetraisopropyldisiloxane-1.3-diyl group was removed by treatment of the protected compound **4**, dissolved in 7 ml of dioxane, with 3.5 ml of 1 M tetrabutylammonium fluoride in tetrahydrofurane. Analysis by TLC ($CHCl_3/CH_3OH$ 95:5 v/v) indicated the complete deprotection within 10 min. The solvent was evaporated and the residue was partitioned between diethyl ether and water. The aqueous phase was concentrated, and the product **5** was obtained either by crystallization from diethyl ether or by purification by column chromatography on silica gel using $CHCl_3$ with a gradient of 0-20 % CH_3OH as eluent. Yield: 0.15 g (0.6 mmol, 60 %) of **5**; m.p. 158 °C; UV λ_{max1} = 244 nm, λ_{max2} = 304 nm, λ_{min1} = 235 nm, λ_{min2} = 263 nm; ^{1}H-NMR (DMSO-d_6): δ [ppm] 2.2-2.6 (m, 2H, H-2'), 3.5-3.6 (m, 2H, H-5'), 3.8 (m, 1H, H-4'), 4.3 (m, 1H, H-3'), 4.9 (t, 1H, J= 5.5, 5'-OH), 5.3 (d, 1H, J=4.0, 3'-OH), 6.2 (dd, 1H, J_1=6.3, J_2=7.4, H-1'), 6.5 (br, 2H, 2-NH_2), 8.3 (s, 1H, H-6), 8.6 (s, 1H, H-8); MS: m/z 251 (M^+, 7.9 %), 135 (base + H^+, 100 %), 136 (base + $2H^+$, 19.6 %), 117 (deoxyribose$^+$, 4.34 %).

Phosphoramidite building block (6). The 5'-O-dimethoxytritylated N^2-isobutyryl protected phosphoramidite **6** of the modified nucleoside **5** was prepared using chloro-(2-cyanoethoxy)-diisopropylamino-phosphine according to standard procedures[10-13]. ^{31}P-NMR (DMSO): δ [ppm] 148.75 (s, 1P, 3'-P), H_3PO_4 (85 %) was used as external standard.

Oligonucleotide synthesis and purification. Oligonucleotides were synthesized by the phosphoramidite method[15] with 2-cyanoethyl as the phosphate protecting group[12]. An automated DNA synthesizer (Gene Assembler Plus, Pharmacia) was used for chain assembly at a 0.2 μmol scale. The modified phosphoramidite building block was dissolved in dry acetonitrile at a concentration of 0.2 M and filtered through 0.45 μmol teflon filters prior to use. Synthesis was performed 'trityl on'; deblocking was performed using 32% aqueous ammonia at 50 °C overnight. Dimethoxytrityl-containing oligonucleotides were purified by HPLC using a RP 18 column (Machery and Nagel 250x13 mm, 3 ml/min) with buffer **A** and a linear gradient of 10 % to 50 % buffer **B** over 40 min. Oligonucleotides containing the dimethoxytrityl group eluted after 25 min. Product containing fractions were evaporated to dryness *in vacuo*, redissolved in 80 % acetic acid (200

µl) and allowed to react at room temperature for 40 min. Acetic acid was removed by evaporation *in vacuo*, followed by coevaporation with water (2x200 µl). The resulting product was dissolved in 500 µl water and extracted with ethyl acetate (3x100 µl). The aqueous layer was evaporated to dryness and the oligomers stored at -20 °C. Yield was about 6 $O.D._{260}$ units per oligonucleotide.

Nucleoside composition analysis. 0.2 $O.D._{260}$ units of each oligonucleotide were dissolved in 6 µl buffer **D**. Snake venom phosphodiesterase (3 µl) and alkaline phosphatase (1 µl) were added and the mixtures were incubated at 37 °C for 3 h. The reactions were analyzed by HPLC using an RP 18 column (125x8 mm, 1 ml/min) with buffer **C** and a gradient of acetonitrile according to the following equation.

$$P_{AN}(t) = P_{AN1} + (P_{AN2} - P_{AN1}) \frac{e^{2t/T} - 1}{e^2 - 1}$$

T = 10 min, P_{AN1} = 0 % CH_3CN, P_{AN2} = 13 % CH_3CN

ACKNOWLEDGEMENT

This research was supported by the Volkswagenstiftung (I/66 393). We are grateful to Prof. H.-J. Fritz for helpful discussions.

REFERENCES

(1) Fox; J.J.; Wempen, I.; Hampton, A.; Doerr, I.L. *J. Am. Chem. Soc.* **1958**, 80 , 1669.
(2) Frederikson, S. *Biochem. Pharm.* **1965**, 14 , 651.
(3) McLaughlin, L.W.; Leong, T.; Benseler, F.; Piel, N. *Nucleic Acids Res.* **1988**, 16 , 5631.
(4) Kung, P.P.; Jones, R.A. *Tetrahedron Lett.* **1991**, 32, 3919.
(5) Stimac, A.; Muhic, D.; Kobe, J. *Nucleosides & Nucleotides* **1994**, 13, 625.
(6) Schmidt, S., Cech, D. *Collect Czech.Chem.Commun.* **1993**, 58, 179.
(7) Robins, M.J.; Wilson, J.S.; Hansske, F. *J.Am. Chem. Soc.* **1983**, 105, 4059.
(8) Gerster, J.F.; Jones, J.W.; Robins, R.K. *J. Org. Chem.* **1963**, 28, 945.
or commercially available from Sigma-Aldrich, Deisenhofen.
(9) Markiewicz, W.T. *J. Chem. Research (S)* **1979**, 24.
(10) Ti, G.S.; Gaffney, B.L.; Jones, R.A. *J. Am. Chem. Soc.* **1982**, (104), 1316.
(11) McBride, L.J.; Caruthers, M.H. *Tetrahedron Lett.* **1983**, 24, 245.
(12) Eritja, R.; Kaplan, B.E.; Mhaskar, D.; Soers, L.C.; Petruska, J.; Goodman, M.F. *Nucleic Acids Res.* **1986**, 14, 5869.
(13) Sinha, N.D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, 12, 4539.

(14) Petrauskene, O.V.; Schmidt, S.; Karyagina, A.S.; Nikolskaya, I.I.; Gromova, E.S.; Cech, D. manuscript submitted for publication

(15) Beaucage; S.L.; Caruthers, M.H. *Tetrahedron Lett.* **1981**, 22, 4539.

Received November 17, 1994
Accepted February 20, 1995