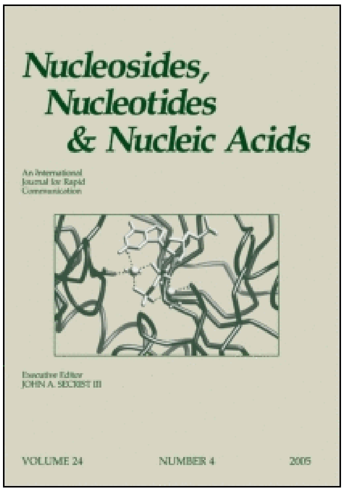

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A SIMPLE ONE-POT METHOD FOR 6-OXOPURINE RIBONUCLEOSIDE SYNTHESIS: CONTROL AND MECHANISM OF ISOMER DISTRIBUTION

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Abstract - Combination of bis(trimethylsilyl)acetamide as a silylating reagent and trimethylsilyl trifluoromethanesulfonate as a catalyst forms an exceptionally stable system for a facile one-pot preparation of 6-oxopurine ribonucleosides. The 7β isomer of the ribonucleosides, formed first, is converted into the 9β isomer presumably via the 7,9-diribonucleoside. The procedure affords both 7β and 9β isomers, but finally yields the 9β isomer as the main product.

INTRODUCTION

The reactions of persilylated heterocyclic bases with per-O-acylated sugars[1] (the silyl-Hilbert-Johnson reaction) or per-N,O'-acylated pyrimidine nucleosides[2] (the transglycosylation reaction) have become standard methods for the synthesis of purine nucleosides. Recently, both procedures have employed highly reactive silylating agents and Friedel-Crafts catalysts like trimethylsilyl perfluoroalkanesulfonates. The use of these reagents simplifies the procedures by combining the several steps of the reaction (silylation of the heterocyclic base, silylation of the catalyst and the nucleoside synthesis itself) into a simple one-step/one-pot reaction.[3-6]

Vorbruggen and Bennua have combined the silylation of both the heterocyclic bases and the catalysts, e.g. triflate or nonaflate acids

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or salts, by the use of mixtures of trimethylchlorosilane (TCS) and hexamethydisilazane (HMDS).[3,4] The procedure gave crystalline adenosine (63%) and guanosine (44%) from N^6-benzoyladenine and N^2-acetylguanine, respectively, in the presence of potassium nonaflate/TCS/HMDS.[4] Unfortunately this particular reaction gave lower yields of the purine nucleosides than the conventional two-step reaction,[7] and was reported to be unsuitable for nucleoside syntheses which are especially sensitive to humidity or inorganic salts.[4] Moreover it is important that all byproducts be neutralized as salts: for example, ammonia would deactivate the catalyst and hydrogen chloride would cause desilylation of the heterocyclic base. The problem of acidic or basic byproducts in the Friedel-Crafts catalyzed silyl-Hilbert-Johnson nucleoside synthesis can be solved by using bis(trimethylsilyl)acetamide (BSA) as silylating reagent, in which case the only likely byproducts are trimethylsilylacetamide (MSA) and acetamide.

The transglycosylation reaction, improved by Azuma and Isono,[2] utilizes the sugar moiety derived from a pyrimidine nucleoside for condensation with the purine bases. It has some advantage in the synthesis of various modified purine ribonucleosides[2,5,6] but introduces in syntheses of unmodified purine nucleosides undesirable pyrimidine derivatives. The success of the one-pot transglycosylation reaction using BSA[5,6] indicates that it is generally useful to prepare silylated bases for condensation reactions using Friedel-Crafts catalysts like trimethylsilyl triflate (TMSTF).

We felt that the advantages of both methods could be combined in a one-pot preparation of purine ribonucleosides. The system TMSTF/BSA used in the transglycosylation reaction can serve as silylating agent and as a practically stable Friedel-Crafts catalyst as long as BSA (and/or MSA) can regenerate the silylated base and TMSTF. Moreover, the commercially available peracetylated sugars can serve as a suitable source of the sugar moiety under these conditions. The above idea was used in our laboratory for the synthesis of 6-oxopurine ribonucleosides.[8]

THE ONE-POT SYNTHESIS OF 6-OXOPURINE RIBONUCLEOSIDES

Our procedure consists of the following steps: (1) silylation of the purine base in acetonitrile with an excess of BSA and (2) the condensation reaction with tetra-O-acetylribofuranose and TMSTF. The steps can be combined in a one-pot procedure because it is not necessary to purify the persilylated base, and the remaining BSA (and/or MSA) serves in the next step as protecting agent for the catalyst, i.e. to prevent inactivation by proton-donor impurities in solvents or starting materials. The byproducts have no effect on the condensation step,[6] whereas other silylating reagents afford acids or bases which have to be removed to obtain good yields of nucleosides.[1]

The above procedure was successfully applied for synthesis of 2-bromohypoxanthine (2-BrHx), guanine and N^2-(*p*-n-butylphenyl)-guanine (BuPG) ribonucleosides which were obtained in over 70% yield. The reaction was carried out in boiling acetonitrile. Typical reaction mixtures after 0.5 h contained only nucleoside products with or without a trace of tetra-O-acetylribofuranose. The products were identified[8] as 7β and 9β ribonucleosides. In each mixture was also present a purine diribonucleoside as a minor component ($\sim$10%) (see below). HPLC analysis of the reaction mixture during the first 0.5 h (see for example FIG. 1) indicated that the 7β isomer is formed first, but that it is converted into the 9β isomer. After approximately 4 h the reaction mixture contained mainly 9β isomer (70-80%) in the presence of the 7β isomer and diribonucleoside. Further prolongation of the reaction did not change the relative concentrations of the components. Simple workup with water/chloroform extraction gave the desilylated O'-acetylated nucleosides and the base, which was removed

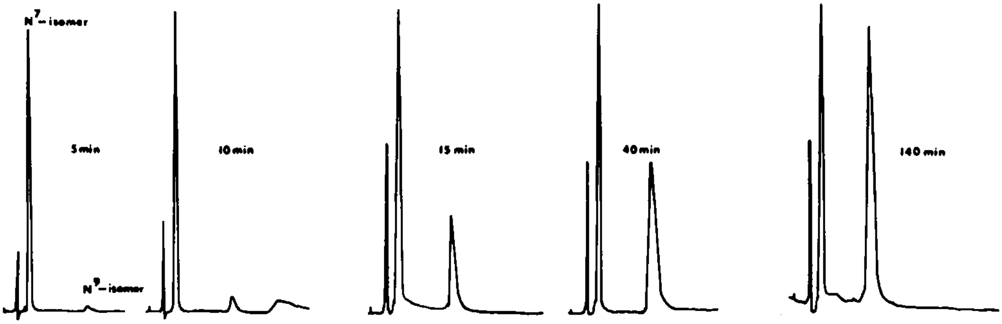

FIGURE 1. HPLC analysis of the reaction mixture during BuPGR synthesis. Initial molar ratio of BuPG: tetra-O-acetylribofuranose, 1.5:1. See Experimental Section for details.

TABLE 1. The One-pot Synthesis of Ribonucleosides

Base	Reaction conditions	Product	Yield [%]	Method of workup
2-BrHx	TMSTF/BSA=1.25/2.0	7β	10	chromatography
	6 h, refluxing MeCN	9β	75	cryst. & chrom.*
BuPG	TMSTF/BSA=1.25/3.0	7β	11	chromatography
	8 h, refluxing MeCN	9β	69	crystallization
N^2-AcG**	TMSTF/BSA=1.25/3.0	7β	5	chromatography
	8 h, refluxing MeCN	9β	70	chromatography

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*About 85% of the 9β isomer present in the reaction mixture can be obtained by direct crystallization. **N^2-AcG, N^2-acetylguanine

from the mixture by filtration. Acetylated BuPG 9β-ribonucleoside (BuPGR) was obtained in crystalline form by direct crystallization from ethanol. The other nucleosides were isolated and purified by column chromatography to give maximum yields of both isomers, and the results are summarized in TABLE 1. Deacetylation by standard methods typically gives free ribonucleosides in 85-99% yields.

CHARACTERIZATION OF 2-BrHx AND BuPG DIRIBONUCLEOSIDES

Our attempts to isolate the minor diribonucleoside components of the reaction mixtures gave good results only in the cases of BuPG and 2-BrHx ribonucleoside syntheses. In each case the diribosides did not exceed 5-10% of total nucleoside products. These products were identified by ^{1}H NMR spectroscopy (TABLE 2). Spectra of both products show the presence of two sets of sugar proton signals, identified by a systematic decoupling procedure, and the existence of only one set of base proton signals as shown by integration. The signal of H8 of the nucleosides exhibited in both cases strong downfield chemical shifts (δ9.3-9.5 ppm) compared to those of the ribosides and rapidly exchanged with deuterium from D_2O at room temperature. These data strongly support a hypothesis that both compounds are 7,9-diribonucleosides that probably exist as zwitterionic forms. For example, 7-methylguanosine, a 7,9-disubstituted purine analog, showed an 8H resonance at δ9.6,

TABLE 2. 250 MHz ^1H NMR Chemical Shifts (δ, ppm) and Coupling Constants (J, Hz) of 7,9-Diribonucleosides

	N^2(*p*-n-Butylphenyl)-7,9-di(β-D-ribofuranosyl)guanine* (Me_2SO-d_6)		2-Bromo-7,9-di(tri-O-acetyl-β-D-ribofuranosyl)hypoxanthine ($CDCl_3$)	
δ_8	9.48 (exch.)		9.29 (exch.)	
δ_{N^2H}	8.91 (exch.)		-	
	A**	B**	A	B
$\delta_{1'}$	6.30	6.01	6.81	6.37
$\delta_{2'}$	4.38	4.51	5.71	5.76
$\delta_{3'}$	4.22	4.24	5.38	5.56
$\delta_{4'}$	4.07	4.16	4.51	4.53
$\delta_{5'}$	3.77	3.77	4.59	4.68
$\delta_{5''}$	3.67	3.66	4.37	4.27
$J_{1',2'}$	3.9	3.7	4.1	5.5
$J_{2',3'}$	4.7	4.7	5.7	5.6
$J_{3',4'}$	5.2	4.1	5.6	2.4
$J_{4',5'}$	3.3	3.3	6.5	6.8
$J_{4',5''}$	3.4	3.2	1.9	2.3
$J_{5',5''}$	-11.8	-12.0	-11.7	-12.2

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*Butylphenyl proton shifts were identical with those of ribosides (ref.8). **A and B represent the individual ribose moieties of each compound.

rapidly exchangeable with D_2O.[9] Also, among a series of isomeric dibenzylhypoxanthines only the 7,9-dibenzyl compound showed a strongly deshielded 8H resonance (δ9.42) compared with those of 1,7- and 1,9-dibenzyl isomers (ca. δ8.5) or hypoxanthine itself (δ8.12).[10] The ability of the diribonucleosides to decay into 7β and 9β isomers is further evidence for their structures (see below). Attempts to isolate the minor polar component during N^2-acetylguanosine synthesis completely failed due its continuous decay during the separation procedure.

STUDY OF THE MECHANISM OF ISOMER DISTRIBUTION

The exceptional uniformity of this reaction gave us an opportunity to investigate some details of the reaction mechanism responsible for 7β and 9β ribonucleoside isomer distribution. We chose the reaction involving BuPG and monitored the distribution of isomers with reaction time using HPLC and TLC.

Each of the 7β and 9β isomers of N^2-(*p*-n-butylphenyl)-guanine ribonucleoside (BuPGR) in the presence of persilylated BuPG reproduced the final reaction mixture after a few hours under the reaction conditions. The 7β isomer after 5 h was converted into the 9β isomer in 70% yield. A similar experiment with the 9β isomer gave only 15% of the 7β isomer after 2 h under the same conditions. These results are evidence for reversibility of the nucleoside synthesis, previously observed for per-O-acylated guanine nucleosides in the presence of mercuric salts.[11] They clearly indicate a continuous sugar exchange in the purine nucleoside synthesis leading to an equilibrium mixture of products. The overall yield of a particular isomer thus depends strongly on the relative stability of the isomer and may vary for different purine bases.

The changes in the ratio of 7β and 9β purine ribonucleoside isomers which we have observed are very characteristic for guanine nucleoside syntheses.[11] Similar changes were also found in the synthesis of the inosine analogs, imidazo[4,5-d]pyridazin-4(5H)-one ribonucleosides.[12] The results indicate that the 7β isomer is clearly kinetically favored in the one-pot reaction conditions (FIG. 1). These findings are apparently in opposition to accepted opinion that purine nucleoside formation proceeds <u>via</u> initial N-3 glycosylation of the purine base.[13] However, faster N-3 → N-7 isomerization than N-3 isomer formation could account for this observation.

2-Bromo-7,9-di(tri-O-acetyl-β-D-ribofuranosyl)hypoxanthine was used to investigate its possible role in the reaction mechanism. This compound, after previous silylation with BSA, was heated in acetonitrile in the presence of TMSTF as the catalyst and persilylated 2-BrHx. After less than 0.5 h, TLC analysis in solvents A, B and C (see Experimental Section) showed the presence of the 9β isomer (60-70%) and the 7β isomer (20-30%). The final reaction mixture after 1 h contained about 80% of 9β riboside, 20% of 7β riboside and no substrate. Therefore, we postulated that the diriboside is an intermediate in N-7 $\rightleftarrows$ N-9 interconversion and that its increased concentration in the reaction mixture should lead to faster interconversion. In order to prove the postulate we treated 2-BrHx under the one-pot conditions with different initial concentrations of tetra-O-acetylribofuranose, and the results of these experiments are summarized by the HPLC analyses in FIG. 2. The reaction with a two-fold excess of sugar showed both faster appearance of ribonucleosides and faster conversion of N-7 → N-9 isomers (FIG. 2B) compared with the reaction in which the base was in excess (FIG. 2A).

CONCLUSIONS

Although the present paper is not an exhaustive study of purine ribonucleoside synthesis, it contains several important points in optimization of the synthesis. The reaction gives smoothly and in a very short time optimal yield of the ribonucleosides as a result of an equilibrium. In the procedure, weak points of the silyl-Hilbert-Johnson condensation reaction are removed because it is not necessary to purify the silylated base and the catalyst is relatively stable due to continuous protection by an excess of BSA. The TMSTF/BSA system permits carrying out the reaction to a desirable isomer ratio without danger of base desilylation or catalyst deactivation due to a small amount of water in the air or the reaction pot. The proposed dependency of N7 → N9 interconversion velocity on diribonucleoside concentration indicates that an excess of sugar reagent can be used

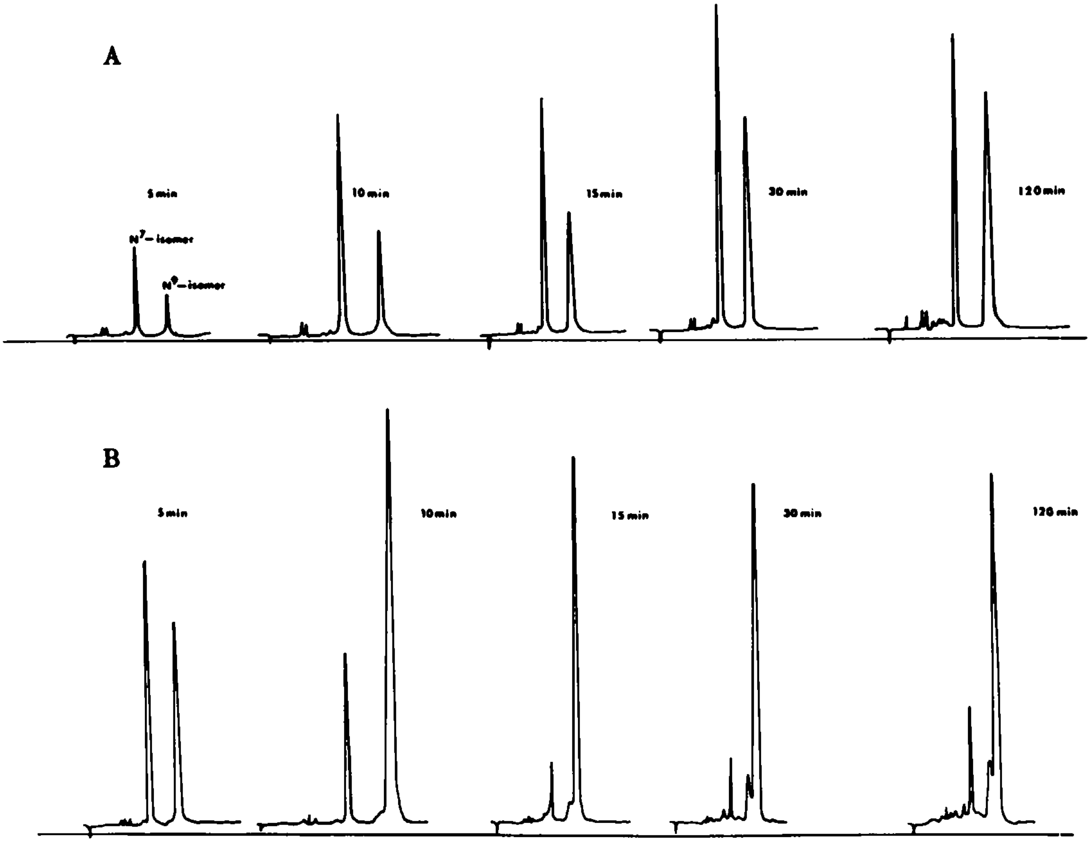

FIGURE 2. HPLC analysis of the reaction mixture during 2-BrHx ribonucleoside synthesis. (A) Initial molar ratio of 2-BrHx: tetra-O-acetylribofuranose, 1.5:1. (B) Initial molar ratio of 2-BrHx: tetra-O-acetylribofuranose, 1:2. See Experimental Section for details.

for especially sensitive substrates, where short time of the reaction is as desirable as high yield of 9$^{\beta}$ isomer.

EXPERIMENTAL SECTION

Bis(trimethylsilyl)acetamide and trifluoromethanesulfonic acid were purchased from Aldrich Chemical Co. Anhydrous acetonitrile was purchased from Matheson Coleman & Bell and used without further purification. Trimethylsilyl trifluoromethanesulfonate was purchased from Fluka AG or prepared according to Marsmann and Horn.[14] All other reagents and solvents including HPLC grade solvents were purchased from Fisher Scientific Co. Preparative column chromatography was performed on E. Merck silica gel 60, 0.04 - 0.06

mm, and all TLC was performed on E. Merck silica gel 60 plates, F-254, developed in solvents: A (methanol:chloroform=5:95), B (methanol: chloroform=1:9) and C (methanol:chloroform:acetic acid=5:95:0.5). Deuterated solvents for NMR were purchased from Aldrich.

HPLC was performed using the following components from Waters Associates, Inc.: Porasil column (3.9x300 mm), 6000A pump, U6K universal injector and 450 UV variable wavelength detector, at a flow rate of 1 mL/min and solvent D (chloroform:methanol:acetic acid=99:1:-0.2). A typical separation time did not exceed 40 min. ^1H NMR spectra were performed using Bruker 250 MHz and Perkin Elmer R12B 60 MHz FT mode instruments with Me_4Si as internal standard. UV spectra were recorded on a Beckman Model 25 spectrophotometer. Elemental analyses were done by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y.

Identification of 6-oxopurine ribonucleosides was fully described in a separate paper.[8] Authentic samples of the compounds were used here as standards for TLC and for calibration of the HPLC column.

The one-pot 6-oxopurine ribonucleoside synthesis. A slurry of 12 mmol of appropriate purine base in 50 mL of dry acetonitrile was treated with an equimolar amount of BSA for each active proton of the base (e.g. for 2-BrHx, 24 mmol of BSA) during 15 min under reflux. To the clear solution of silylated base 50 mL of a solution of tetra-O-acetylribofuranose (10 mmol) in acetonitrile was added, followed by addition of 15 mmol of TMSTF. [The tetra-O-acetylribofuranose has to be carefully dried (60°C, 0.02 mmHg, P_2O_5 as drying agent).] Complete conversion of the sugar into nucleoside components is found after 0.5 h under reflux. For maximum yield of the 9β isomer it is necessary to continue the reaction for several hours (usually 4-8 h). The reaction is quenched by cooling to room temperature. After evaporation of the reaction mixture the syrupy residue is treated with a chloroform:water mixture (300 mL:150 mL). The cloudy biphasic mixture is filtered to remove unreacted base, and the organic phase is separated by filtration using Whatman phase separator, washed several times with water to pH 6 of the aqueous phase, and dried with anhydrous sodium sulfate. The 9β isomers of 2-BrHx and BuPG may be obtained by simple crystallization of the residue from chloroform extracts (from MeOH or EtOH). Isolation and purification of other isomers was done by preparative column chromatography in solvent C or D. TABLE 1 summarizes the details for each base.

Ribonucleoside synthesis in an excess of purine base. A solution of tetra-O-acetylribofuranose (1 mmol) in acetonitrile (5 mL) and 1.5 mmol of TMSTF were added to a solution of base (1.5 mmol) which had been silylated with 2 mL of BSA (final volume of the reaction mixture 11 mL). Samples (1 mL) of the reaction mixture were removed after 5, 10, 15, 30 and 120 min and each time the reaction was quenched by the addition of a mixture of chloroform and water (10 mL: 10 mL). Each sample was filtered, and the organic phase was collected by filtration through phase separator and dried. Each extract was analyzed by HPLC. Independently each sample before and after work-up was analyzed by TLC.

Ribonucleoside synthesis in an excess of the sugar. The procedure was similar to the above except that 1 mmol of the base and 2 mmol of the sugar were used. Work-up and analysis of the reaction mixture were done as described above.

Interconversion of BuPG ribonucleosides. In separate experiments pure 7β and 9β isomers of BuPG per-O-acetylribonucleosides (0.2 mmol) in the presence of an equimolar amount of BuPG were silylated in acetonitrile (5 mL) with a ten-fold excess of BSA. The reactions were initiated with 0.4 mmol of TMSTF and the reaction mixtures were analyzed by TLC in solvents A and C after 1, 3 and 5 h.

Decomposition of 2-bromo-di-7,9-(tri-O-acetyl-β-D-ribofuranosyl)hypoxanthine. This diribonucleoside (0.1 mmol) and 2-BrHx (0.2 mmol) were dissolved in acetonitrile (1 mL) and silylated with BSA (2 mmol). TMSTF (0.25 mmol) was added, and the refluxing reaction mixture was analyzed by TLC in solvents A, B and C after 15, 30 and 60 min.

2-Bromo-7,9-di(tri-O-acetyl-β-D-ribofuranosyl)hypoxanthine was isolated from the standard one-pot reaction mixture with 2-BrHx after approx. 1 h of reflux by silica gel chromatography with solvent A. Further purification of the fluorescent compound was done by crystallization from ethanol with a slight amount of water, m.p. 116-120^{o} C. UV (MeOH) pH 1 λ_{max} 255 nm; pH 11 λ_{max} 278 nm and in pure MeOH λ_{max} 272 nm. Anal. Calcd for $C_{27}H_{31}O_{15}N_4Br \cdot 0.5\ H_2O$: C, 43.72%; H, 4.32%. Found: C, 43.68%; H, 4.11%. Further characterization of the compound was done by ^{1}H NMR spectroscopy (see TABLE 2).

N^2-(p-n-Butylphenyl-7,9-di(β-D-ribofuranosyl)guanine. This compound was obtained from reaction mixtures involving BuPG after 1 h. The acetylated derivative isolated as a syrup by silica gel chromatography using solvent A, was deblocked with sodium methoxide in methanol to give the free diribonucleoside. The compound was purified by crystallization from aqueous ethanol. UV pH 1 λ_{max} 272 nm; pH 7 λ_{max} 265 nm (very broad peak); pH 13 λ_{max} 270 nm (decomposition product(s)). Further characterization of the diribonucleoside was done by ^{1}H NMR spectroscopy (see TABLE 2). No elemental analysis was done for the compound due to slight contamination by BuPG 9β-ribonucleoside, not removable because of slow decomposition of the diribonucleoside during chromatography and recrystallization.

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