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Synthesis and hydrolysis of oligodeoxyribonucleotides containing 2-aminopurine

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ABSTRACT

A new method is reported for the synthesis of oligodeoxyribonucleotides containing 2-aminopurine residues at selected sites. This method involves protection of the 2-aminopurine ribonucleoside, reduction to the deoxyribonucleoside and standard preparation of the 5′-*O*-(4,4′-dimethoxytrityl)-3′-*O*-(2-cyanoethyl)-*N*,*N*-diisopropylphosphoramidite. The 2-aminopurine phosphoramidite prepared by this method couples with high efficiency and is stable under standard automated synthesis conditions. The presence and location of the 2-aminopurine residue is easily verified by treatment of the oligodeoxyribonucleotide with hot piperidine. The mechanism for selective hydrolysis of the 2-aminopurine residue in alkaline solution is predominantly direct cleavage of the glycosidic bond.

INTRODUCTION

The adenine analog 2-aminopurine has been studied extensively because of its capacity to generate genetic mutations in procaryotic systems (1–3). More recently oligonucleotides containing 2-aminopurine have been used to study nucleic acid dynamics (4–7). Unlike the normal DNA bases, 2-aminopurine is fluorescent at neutral pH (8) and this native fluorescence has been demonstrated to be an extremely useful probe of DNA conformational changes.

Oligonucleotides containing 2-aminopurine were first synthesized using solid phase chemistry by Eritja *et al.* (9). In this method 2-aminopurine-2′-deoxyribonucleoside was prepared enzymatically from the free base efficiently, but in small scale (10,11). Conversion to the amine-protected derivative, however, proceeded with low yield, due to the low nucleophilicity of the 2-amino group, coupled with enhanced lability of the glycosidic linkage. A fully chemical synthesis has been reported by McLaughlin *et al.* (12), in which deoxyguanosine is converted to 2-aminopurine-2′-deoxyribonucleoside via nitration and reduction. In our hands this method proved difficult, because of the large excess of reagents required and the complexity of the products formed.

We describe in this paper a new chemical method which provides the necessary phosphoramidite cleanly and in high yield. The phosphoramidite produced by this method has been used to produce 2-aminopurine-containing oligodeoxyribonucleotides for several recent biophysical studies. These studies include the development of a method to assay DNA helicase activity (4), observation of the melting of correct and incorrect base pairs in the active site of DNA polymerase (5), and a spectroscopic method to probe nucleotide incorporation by DNA polymerase (6).

Recently Schmidt and Cech (13) presented a similar method for the production of a 2-aminopurine phosphoramidite based upon the reduction of 6-chloroguanosine to 2-aminopurine-2′-deoxyribonucleoside. In the procedure reported here, however, protection of the 2-amino group occurs prior to reduction of the sugar moeity, thus exploiting the enhanced stability of the glycosidic linkage of ribonucleosides and circumventing the known instability of 2-aminopurine-2′-deoxyribonucleoside to acylation conditions (9).

In addition to the synthetic method, we report on the hydrolysis of 2-aminopurine-containing oligodeoxyribonucleotides under acidic and alkaline conditions. An understanding of the conditions which result in the hydrolysis of 2-aminopurine deoxyribonucleoside derivatives is necessary in order to prevent degradation of oligodeoxyribonucleotides following synthesis, as well as to develop methods to establish the presence and location of 2-aminopurine residues in synthetic oligodeoxyribonucleotides.

MATERIALS AND METHODS

Materials

Thioguanosine (2-amino-6-mercaptopurine riboside), Raney nickel, isobutyryl chloride, 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane, 4-dimethylaminopyridine (DMAP), phenyl chlorothionoformate, tributyltin hydride, tetrabutylammonium fluoride, 4,4′-dimethoxytrityl chloride, 2-cyanoethyl-*N*,*N*-diisopropylchloro-phosphoramidite, methanol, dichloromethane, pyridine, toluene and silica gel were obtained from Aldrich. AIBN [2,2′-azobis(2-methylpropionitrile)] was obtained from Kodak. Silica gel H was obtained from Fluka.

HPLC was performed with a Perkin-Elmer Series 4 liquid chromatograph interfaced with an LKB 2140 spectral diode array detector. UV spectra were recorded with a Perkin-Elmer Lambda 3B UV/Vis spectrophotometer. GC/MS analysis was performed with a Hewlett Packard 5890 gas chromatograph interfaced with a 5970 Series mass selective detector. NMR spectra were

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recorded with a Varian Unity 300 spectrometer. DNA synthesis was performed with a Pharmacia Gene Assembler.

Preparation of the 2-aminopurine phosphoramidite

*2-Aminopurine ribonucleoside (**1**).* Thioguanosine was reduced with Raney nickel in boiling water as described previously (14,15). The progress of the reaction was monitored by TLC. Upon reduction the 2-aminopurine ribonucleoside is observed as a blue fluorescent spot. The resulting solution was filtered and the solvent was removed under reduced pressure. The residue was taken up into dichloromethane/methanol and the product, 2-aminopurine ribonucleoside, was isolated by silica gel chromatography. Yield, 91%; ^{1}H NMR (300 MHz, d_6DMSO) δ, 8.60 (s, H8), 8.30 (s, H6), 6.40 (s, NH_2), 5.83 (d, H1′,*J* = 6.0 Hz), 5.44 (d, 2′OH), 5.16 (d, 3′OH), 5.06 (t, 5′OH), 4.51 (m, H2′), 4.12 (m, H3′), 3.38 (m, H4′), 3.69–3.49 (m, H5′,H5″).

*N-Isobutyryl-2-aminopurine ribonucleoside (**2**).* The transient protection scheme of Ti *et al*., using trimethylsilyl chloride followed by isobutyryl chloride, was then applied (16). The solvent was evaporated under reduced pressure and the residue was separated by silica gel chromatography. Yield, 70%; ^{1}H NMR (300 MHz, d_6DMSO) δ, 10.62 (s, NH) 9.01 (s, H8), 8.68 (s, H6), 5.96 (d, H1′,*J* = 6.0 Hz), 5.55 (d, 2′OH), 5.22 (d, 3′OH), 4.97 (t, 5′OH), 4.65 (m, H2′), 4.20 (m, H3′), 3.95 (m, H4′), 3.71–3.49 (m, H5′,H5″), 2.83 (m, C<u>H</u>$(CH_3)_2$), 1.10 (d $(CH_3)_2$).

*N-Isobutyryl-2-aminopurine-2′-deoxyribonucleoside (**3**).* The selective deoxygenation scheme of Robins *et al*. (17) was applied to the reduction of 2-aminopurine ribonucleoside. The 3′ and 5′ hydroxyls of 2-aminopurine ribonucleoside were protected with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine. The progress of the reaction was monitored by TLC. Complete conversion to a single product spot was observed in 3 h. Solvent was evaporated and the residue was washed with cold 1.0 N HCl, saturated bicarbonate and partitioned between brine and ethyl acetate. The organic layers were combined and concentrated.

The silylated product was used directly in the next step. The 2′-hydroxyl was converted using phenyl chlorothionoformate and DMAP in acetonitrile. Complete conversion was observed by TLC. Solvent was removed under reduced pressure and the product was washed and partitioned between brine and ethyl acetate. The organic layer was separated and evaporated under reduced pressure and used directly in the subsequent reaction.

Reduction of the 3′-phenoxythiocarbonyl intermediate was achieved with tributyltin hydride in toluene containing AIBN under an argon atmosphere. After 3 h at 75°C the reduction was complete, as judged by TLC analysis. Silyl groups were removed *in situ* using tetrabutylammonium fluoride. The solvent was evaporated and the product, *N*-isobutyryl-2-aminopurine-2′-deoxyribonucleoside, was isolated by silica gel chromatography. The overall yield from the ribo to the deoxyribo derivative was 72%. ^{1}H NMR (300 MHz, d_6DMSO) δ, 10.58 (s, NH) 8.98 (s, H8), 8.66 (s, H6), 6.39 (t, H1′,*J* = 6.76 Hz), 5.34 (d, 3′OH), 4.89 (t, 5′OH), 4.44 (m, H3′), 3.86 (m, H4′), 3.65–3.47 (m, H5′,H5″), 2.87–2.71 (m, H2′,H2″), 2.31 (m, C<u>H</u>$(CH_3)_2$), 1.09 (d $(CH_3)_2$).

*N-Isobutyryl-2-aminopurine-2′-deoxyribonucleoside-5′-O-(4,4′-dimethoxytrityl)-3′-O-(2-cyanoethyl)-N,N-diisopropylphosphoramidite (**4**).* The *N*-isobutyryl-2-aminopurine-2′-deoxyribonucleoside was converted to the 2-aminopurine phosphoramidite by standard methods (18). Dimethoxytrityl was added to the 5′ hydroxyl group using 4,4′-dimethoxytrityl chloride in pyridine. The product was washed with brine, extracted with ethyl acetate and purified by silica gel chromatography. The purified trityl derivative was converted to the phosphoramidite using 2-cyanoethyl-*N,N*-diisopropylchlorophosphoramidite and diisopropylethylamine in dry acetonitrile and isolated using silica gel H.

Oligonucleotide synthesis and purification

The 2-aminopurine phosphoramidite was dissolved in dry acetonitrile and placed in one of the additional ports of the DNA synthesizer. A controlled pore glass resin containing fluorescein (fluorescein-CPG; Glenn Research) was loaded into an empty synthesis cartridge. The standard 1.3 µmol synthesis cycle with retention of the 5′-terminal dimethoxytrityl group was used without modification. The sequence of the oligonucleotide was 5′-AAA CCC ATG TPC GGC TGC GAF-3′, where P is 2-aminopurine and F is fluorescein.

Following synthesis the solid support was placed in concentrated aqueous ammonia in a sealed vial and heated at 65°C overnight. The solution was cooled, filtered and a fraction was taken directly for gel analysis. The trityl-containing oligonucleotide was purified by HPLC on a Hamilton PRP semipreparative column using 0.1 M triethyl ammonium acetate and an acetonitrile gradient. The purified trityl-containing oligonucleotide was detritylated in 80% acetic acid at room temperature for 30 min. The aqueous acetic acid was removed under reduced pressure. The residue was dissolved in water, the pH was adjusted to 7 and extracted with ethyl acetate. The aqueous phase containing the detritylated oligonucleotide was concentrated under reduced pressure.

Gel electrophoresis of the oligonucleotide

The fluorescein-labeled 2-aminopurine-containing oligonucleotide was analyzed by gel electrophoresis on a 20% polyacrylamide denaturing gel. Approximately 1 pmol fluorescein oligonucleotide was loaded in each gel lane. Bands were visualized and photographed by placing the gel directly on a UV transilluminator. Band intensities were measured by scanning with a CCD camera and quantitation using RFLPscan software.

Analysis of base composition

The purified oligonucleotide was enzymatically digested using nuclease P1 and bacterial alkaline phosphatase (19). The liberated deoxyribonucleosides were separated by HPLC using a reverse phase column and a gradient of 0.05 M sodium phosphate, pH 4.0, against methanol (0–20%). Detection was provided by a photodiode array detector. All four bases, including 2-aminopurine-2′-deoxyribonucleoside, are chromatographically separable and identifiable based upon their characteristic UV spectra. Retention times and molar absorbtivity (pH 4.0, 280 nm) are: dC, 1.8 min, 11.7×10^3; dG, 4.3 min, 7.8×10^3; dT, 5.2 min, 5.9×10^3; dAP, 6.7 min, 2.5×10^3; dA, 8.0 min, 2.6×10^3. All deoxyribonucleosides were present in the correct ratios based upon integration of the HPLC chromatogram at 280 nm.

The oligonucleotide was also acid hydrolyzed to liberate the constituent free bases. The bases were silylated and analyzed by GC/MS as previously described (20). Bases were identified by their characteristic retention times and mass spectra. Observed

retention times for the silylated derivatives were: thymine, 9.9 min; cytosine, 11.5 min; 2-aminopurine, 15.2 min; adenine 15.9 min; guanine, 18.1 min. Solutions of known concentration of the free bases were used to generate standard curves relating base concentration to integrated chromatogram peak areas at appropriate mass ion currents. Based upon these standard curves we were able to verify that all bases were present in synthetic oligonucleotides in the correct ratios.

Restriction enzyme cleavage of the oligonucleotide

The oligonucleotide was annealed with its unlabeled complementary sequence in approximately 1× *Rsa*I enzyme buffer by heating at 90°C, followed by slow cooling. Restriction enzyme (20 U) was added and the reaction was allowed to proceed overnight at room temperature. The oligonucleotide was precipitated with ethanol, resuspended in the loading buffer and analyzed by gel electrophoresis.

Hydrolysis of the oligonucleotide in piperidine

The fluorescein-labeled 2-aminopurine-containing oligodeoxyribonucleotide was treated with 1.0 M piperidine at 90°C, essentially as described by Pless and Bessman (21), for 1, 12 and 24 h. The solution was concentrated under reduced pressure and added directly to the gel loading buffer. The reaction mixture was heated briefly and analyzed by gel electrophoresis.

Hydrolysis of 2′-deoxyadenosine and 2-aminopurine-2′-deoxyribonucleoside

The hydrolysis of 2′-deoxyadenosine and 2-aminopurine-2′-deoxyribonucleoside in acid solution was monitored by measuring changes in the UV spectrum of solutions of each nucleoside in 0.1 M HCl as functions of time and temperature, as described by Garrett and Mehta (22). 2′-Deoxyadenosine hydrolysis was measured at 258 nm, whereas for 2-aminopurine-2′-deoxyribonucleoside the reaction was monitored at 310 nm. In acid solution, changes in the UV spectra were consistent with free base release upon cleavage of the glycosidic bond. Hydrolytic ring opening of the purine ring in acid solution was not observed. The rate constant for each hydrolysis reaction was measured by plotting the change in UV absorbance as a function of time. The activation energy for hydrolysis was determined by measuring the reaction rate constant at several temperatures and plotting the reciprocal of the absolute temperature versus the apparent rate constant.

Hydrolysis of the deoxyribonucleosides in 1.0 M piperidine was measured simultaneously by analysis of reaction products by GC/MS. A solution was prepared containing 2′-deoxyadenosine, 2-aminopurine-2′-deoxyribonucleoside and thymine (as an internal standard). A portion of this solution was acid hydrolyzed in formic acid and analyzed by GC/MS to determine the peak sizes for each base upon complete hydrolysis of the glycosidic bond of the purine deoxyribonucleosides. Another portion of the nucleoside and thymine solution was added to 2 M piperidine to generate a final piperidine concentration of 1 M. This solution was heated at 90°C and portions were removed, cooled and evaporated as a function of time. The reaction products were silylated and analyzed by GC/MS.

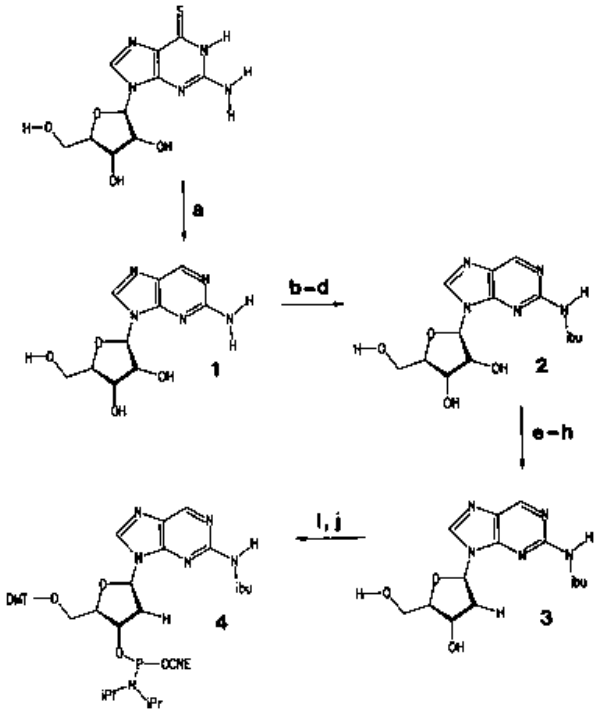

Figure 1. Synthetic scheme for preparation of the 2-aminopurine phosphoramidite. (a) Raney Ni/H_2O; (b) trimethylsilyl chloride/pyridine; (c) isobutyryl chloride/pyridine; (d) NH_4OH; (e) 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane/pyridine; (f) phenyl chlorothionocarbonate/4-*N*,*N*-dimethylaminopyridine/acetonitrile; (g) tri-*n*-butyltin hydride/2,2′-azobis(2-methylpropanitrile)/toluene, 75°C; (h) tetra-*n*-butylammonium fluoride; (i) 4,4′-dimethoxytrityl chloride/pyridine; (j) 2-cyanoethyl-*N*,*N*-diisopropylchlorophosphoramidite/diisopropylethylamine/acetonitrile.

RESULTS AND DISCUSSION

A new method is here described for the preparation of a 2-aminopurine phosphoramidite suitable for solid phase DNA synthesis. In this method the 2-amino group of the ribonucleoside is protected (Fig. 1). The two primary advantages of this approach are that the ribonucleoside is readily available in one chemical step from a commercially available starting material and that the glycosidic linkage of the ribonucleoside is substantially less labile than that of the corresponding deoxyribonucleoside. In the original procedure of Eritja *et al.* (9) the deoxyribonucleoside was generated in small quantities using an enzyme which is not commercially available. Further, 2-aminopurine derivatives are not efficiently acylated by anhydrides and the use of acid chlorides results in near quantitative cleavage of the 2-aminopurine-2′-deoxyribonucleoside glycosidic bond. The method reported here therefore overcomes both of these original difficulties.

The *N*-protected ribonucleoside is cleanly and efficiently converted to the deoxyribonucleoside by selective deoxygenation of the 2′-hydroxyl group using the method developed by Robins and co-workers (17). The ribonucleoside to deoxyribonucleoside conversion pathway has been utilized successfully in other laboratories for the preparation of other difficult purine nucleosides (23,24). The *N*-protected 2-aminopurine-2′-deoxyribonucleoside was then converted to the 5′-dimethoxytrityl-3′-cyanoethylphosphoramidite by established methods (20).

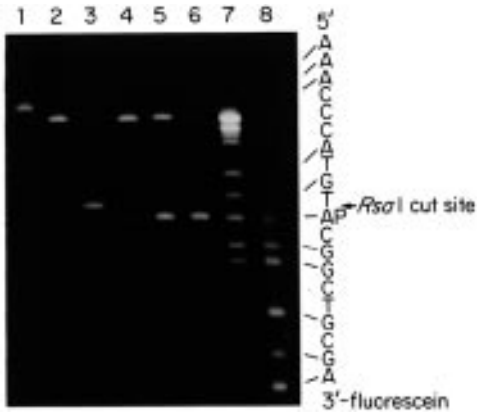

Figure 2. Polyacrylamide gel of the 3′-fluorescein-labeled 2-aminopurine-containing oligonucleotide. Lane 1, crude DMT-containing oligonucleotide prior to purification; lane 2, purified detritylated oligonucleotide; lane 3, oligonucleotide fragment obtained following digestion with *Rsa*I restriction endonuclease; lanes 4–6, 2-aminopurine oligonucleotide heated at 90°C in 1.0 M piperidine for 1, 12 and 24 h respectively; lane 7, oligonucleotide treated with formic acid for 1 h at room temperature; lane 8, oligonucleotide boiled in unbuffered water for 1 h.

The 2-aminopurine phosphoramidite generated by the above method was then used for oligonucleotide synthesis using a commercially available DNA synthesizer. In order to determine coupling efficiency, as well as to probe for potential oligonucleotide cleavage during synthesis, a 2-aminopurine-containing oligonucleotide was synthesized using a fluorescein solid support. Following standard deprotection in ammonia the crude trityl-containing oligonucleotide was examined on a denaturing 20% polyacrylamide gel. When viewed on a UV transilluminator (Fig. 2, lane 1) a single fluorescent band is observed. This demonstrates that the 2-aminopurine phosphoramidite coupled with high efficiency, consistent with trityl release measured during synthesis, and that hydrolysis of the 2-aminopurine residue does not occur to any measurable extent during the standard synthetic cycle. Use of the 3′-fluorescein label ensured that we could visualize both failure and cleaved sequences.

The DMT-containing oligonucleotide was separated by HPLC using a PRP column, aqueous triethylammonium acetate and acetonitrile. The purified oligonucleotide was then detritylated using 80% aqueous acetic acid. The purity of the oligonucleotide is demonstrated in lane 2 of Figure 2. Surprisingly, the 2-aminopurine-containing oligonucleotide was not measurably cleaved under standard detritylation conditions. The base composition of the oligonucleotide was verified by enzymatic digestion followed by HPLC analysis of the liberated deoxyribonucleosides, as well as by acid hydrolysis followed by base analysis using GC/MS (see Materials and Methods).

In order to verify the integrity of the oligonucleotide in the vicinity of the 2-aminopurine residue the sequence of the oligonucleotide was designed to include the recognition site for *Rsa*I restriction endonuclease. *Rsa*I was chosen because it has been previously demonstrated that replacement of adenine by 2-aminopurine in the cut site (GT↑AC) does not inhibit enzymatic cleavage (25). The 3′-fluoresceinated 2-aminopurine-containing oligonucleotide was annealed with its unlabeled complement and cleaved with *Rsa*I. As shown in Figure 2, lane 3, the oligonucleotide is quantitatively cleaved generating one labeled product band of the expected size. The labeled band contains fluorescein on the 3′-end and 2-aminopurine-2′-deoxyribonucleoside-5′-phosphate on the 5′-end.

Previously Pless and Bessman (21) demonstrated that oligodeoxyribonucleotides containing 2-aminopurine could be selectively cleaved using hot piperidine. We applied this method (1.0 M piperidine, 90°C) to our oligonucleotide and the results are shown in lanes 4–6 of Figure 2, representing treatment for 1, 12 and 24 h respectively. Hot piperidine selectively cleaves the oligonucleotide at the position of the 2-aminopurine residue with an apparent half-life of ~12 h. Based upon integration of the fluorescent gel bands the 2-aminopurine residue is cleaved ~15 times more rapidly than either adenine or guanine residues, consistent with the results of Pless and Bessman (21). The resulting labeled fragment is one base shorter than that generated with the restriction endonuclease, because the chemical treatment results in destruction of the 2-aminopurine residue followed by β-elimination. Hot piperidine treatment is therefore an extremely useful method for verifying the presence and position of 2-aminopurine in synthetic oligodeoxyribonucleotides.

In order to verify the sequence of the oligonucleotide it was subjected to the standard Maxam–Gilbert formic acid purine cleavage reaction (26). As shown in Figure 2, lanes 7 and 8, the positions of all purines, including 2-aminopurine, were verified by formic acid treatment at room temperature (lane 7) or boiling for 1 h in unbuffered water (lane 8). Consistent with the results obtained after detritylation in acetic acid, formic acid treatment of the 2-aminopurine-containing oligonucleotide does not result in selective cleavage at the position of the 2-aminopurine residue.

The absence of selective cleavage of the 2-aminopurine residue in acid solution was somewhat surprising, because of the significant lability of the glycosidic bond encountered during preparation of the protected deoxyribonucleoside. We therefore measured the rate and activation energy for hydrolysis of 2-aminopurine-2′-deoxyribonucleoside in 0.1 M HCl according to the methods previously reported for hydrolysis of 2′-deoxyadenosine (22). We measured the rate constant for cleavage of the glycosidic bond to be 8.3×10^{-5}/s at 20°C, with an activation energy of 24.0 kcal/mol. Garett and Mehta (22) reported a somewhat faster rate for hydrolysis of 2′-deoxyadenosine, 12.4×10^{-5}/s, with essentially the same activation energy, 23.2 kcal/mol. The lability of 2-aminopurine-2′-deoxyribonucleotide during protection must therefore derive from cleavage of the protected nucleoside. Following hydrolysis of the *N*-protecting group in ammonia the 2-aminopurine residue in DNA is as stable in acid solution as either adenine or guanine.

In hot piperidine, however, the 2-aminopurine residue is selectively labile. Previously it has been shown that adenosine degrades in alkaline solution via two distinct pathways (27). One is direct displacement of the base with cleavage of the glycosidic bond. The other involves ring opening of the imidazole ring followed by further degradation of the resulting formamidopyrimidine. The relative rates of these two pathways are a complex function of solution pH and temperature.

We considered the possibility that the enhanced lability of 2-aminopurine residues in alkaline solution may in part result from selective ring opening (Fig. 3). It is known that purine ribonucleosides undergo rapid ring opening, even at room temperature (28). Hydrolysis of the imidazole ring of purines generating the corresponding formamidopyrimidine derivative is of interest because such ring opened derivatives may also result from purine oxidation (29,30). Methods to generate ring opened purines in

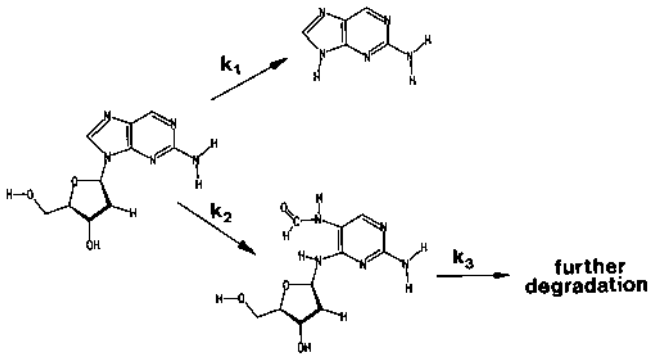

Figure 3. Competing pathways for the hydrolysis of 2-aminopurine-2′-deoxyribonucleoside in alkaline solution.

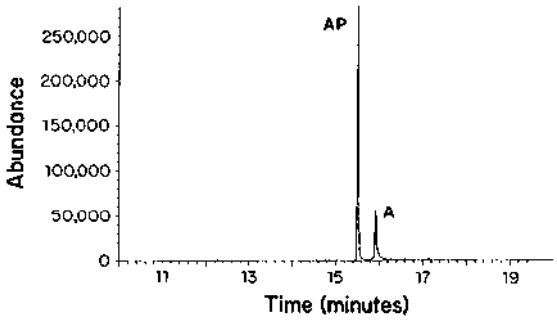

Figure 4. Selected ion chromatograph (264 a.m.u.) of 2-aminopurine (left peak) and adenine (right peak) released from the corresponding deoxyribonucleosides following treatment in 1.0 M piperidine for 2 h.

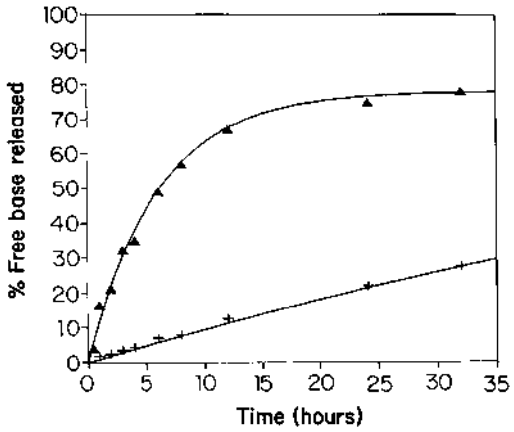

Figure 5. Release of 2-aminopurine and adenine from the corresponding deoxyribonucleosides during treatment with 1.0 M piperidine at 90°C as a function of time.

oligonucleotides at selected positions would be valuable for the preparation of substrates for studies with DNA repair enzymes (31), however, successful synthetic attempts have not yet been reported.

We therefore measured the rates of imidazole ring opening and glycosidic bond cleavage for 2-aminopurine-2′-deoxyribonucleoside and 2′-deoxyadenosine by measuring purine base release under the conditions used for oligonucleotide cleavage. Unlike the parent nucleosides, purine bases are stable in alkaline solution because the N9 proton is acidic and the corresponding conjugate base resists hydrolytic attack even under strongly alkaline conditions (26). Because of the stability of the free base, the rates of hydrolysis of purine nucleosides via the competing pathways can be determined in alkaline solution. We prepared a solution containing equimolar 2′-deoxyadenosine and 2-aminopurine-2′-deoxyribonucleoside in 1.0 M piperidine. The reaction was heated at 90°C and the progress of hydrolysis was followed by GC/MS. Adenine and 2-aminopurine are structural isomers and therefore the silylated derivatives have identical mass (279 a.m.u.) and mass spectra. The two derivatives are chromatographically resolvable, with the adenine derivative more strongly retained (Fig. 4). The rate equations for the generation of 2-aminopurine (AP) from 2-aminopurine-2′-deoxyribonucleoside (APdR) and adenine (A) from 2′-deoxyadenosine (dA) were found to be

$$[AP]_t = [APdR]_o \times (k_1/k_1 + k_2) \times (1 - e^{-(k_1 + k_2) \times t}) \quad \mathbf{1}$$

$$[A]_t = [dA]_o \times (1 - e^{-kt}) \quad \mathbf{2}$$

The values of the rate constants in 1.0 M piperidine, 90°C are

$k_1 = 4.7 \pm 0.2 \times 10^2$/s
$k_2 = 1.3 \pm 0.2 \times 10^2$/s
$k = 3.6 \pm 0.1 \times 10^1$/s

The rate constants k_1 and k_2 correspond to glycosidic bond cleavage and purine ring opening, as shown in Figure 3.

We observed that 2-aminopurine free base was generated significantly faster than adenine (Fig. 5). The difference in the rates of deoxyribonucleoside hydrolysis ($k_1 + k_2$ for APdR) is ~16, similar to the difference in reactivity in the oligonucleotide, although both deoxyribonucleosides are degraded approximately three times faster in solution than in the oligonucleotide. We observed that 78% of the 2-aminopurine-2′-deoxyribonucleoside was converted to the free base. The remaining 22% underwent degradation via ring opening.

The enhanced hydrolytic lability of 2-aminopurine derivatives in alkaline solution therefore results primarily from direct cleavage of the glycosidic bond, although imidazole ring opening of the 2-aminopurine residue is still several times faster than hydrolysis of the glycosidic bond of 2′-deoxyadenosine. It is not immediately obvious why the glycosidic bond of 2-aminopurine residues is significantly more labile than that of adenine resides, in that the presumed hydroxyl attack occurs at the C1′ position and not in the purine moiety itself. A potential explanation may derive from the fact that 2-aminopurine is an order of magnitude more water soluble than adenine (32). Perhaps 2-aminopurine residues are differentially or more extensively hydrated, allowing for more efficient hydrolytic attack on the glycosidic lineage.

CONCLUSIONS

We report here a convenient method for the preparation of a phosphoramidite derivative of 2-aminopurine suitable for automated solid phase DNA synthesis. Preparation of the protected ribonucleoside followed by reduction to the deoxyribonucleoside overcomes several of the previous limitations encountered with preparation of the desired phosphoramidite. The 2-aminopurine phosphoramidite thus prepared couples with high efficiency and no degradation of the 2-aminopurine residue is observed during the standard synthetic cycle. The base composition of oligonucleotides containing 2-aminopurine may be analyzed by HPLC or GC/MS

methods, however, selective cleavage in hot piperidine is demonstrated to confirm both the presence and location of the 2-aminopurine residue.

Destruction of 2-aminopurine residues under alkaline conditions results predominantly from direct cleavage of the glycosidic bond, releasing 2-aminopurine free base. The lability of 2-aminopurine residues to hydrolysis may result from significantly altered hydration, consistent with the enhanced water solubility of the 2-aminopurine free base. The spontaneous release of purines from DNA, generating abasic sites, is considered to be one of the most important mechanisms for induction of spontaneous transversion mutations (34). The data presented here suggest that depurination of 2-aminopurine residues in DNA would be expected to occur faster than depurination of adenine or guanine residues, even under physiological conditions. Transversion mutations, known to be induced by 2-aminopurine (1,2), may therefore arise due to enhanced depurination of 2-aminopurine residues in DNA templates.

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