An Improved Synthesis of [Amino-^{15}N]Adenine; Useful in the Large Scale Synthesis of 2'-Deoxy[Amino-^{15}N]Adenosine.

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SUMMARY

Summary: 2'-Deoxy[Amino-^{15}N]Adenosine has been constructed in two steps from commercially available starting materials. These reactions have been scaled up to give 5 gram lots of labeled material.

Keywords: [Amino-^{15}N]Adenine, 2'-Deoxy[amino-^{15}N]adenosine.

INTRODUCTION

The synthesis of ^{15}N labeled nucleic acids has seen increased utility for the probing of local interactions in polynucleotides by multinuclear and multidimensional nuclear magnetic resonance spectroscopy[2]. Interest in these labeled molecules has been stimulated by new methods which allow for the generation of significant quantities of labeled nucleosides and, as a consequence, synthetic DNA oligomers. Most notable, have been the recent accomplishments of Jones and co-workers in the synthesis of a series of singly ^{15}N labeled 2'-deoxyadenosines and 2'-deoxyguanosines[3]. Their approach, as well as that of Roy *et al.*[4], has allowed for the simple and straightforward construction of large quantities of these materials. We have been interested in the construction of purine derived 2'-deoxynucleosides which are specifically labeled at multiple posititions. To accomplish this task our approach required the synthesis of a large quantity of 2'-deoxy[amino-^{15}N]adenosine, which we viewed as a common precusor, for a series of multiply labeled purine 2'-deoxynucleosides as illustrated in Scheme 1. For example, conversion of **1** to the N-oxide, followed by treatment with [^{13}C,^{15}N]cyanogen bromide, and performance of the Dimroth rearrangement will allow access to **3**[5]. Alkylation of N-1, followed the Dimroth rearrangement and removal of the alkyl group, gives **2**[5].

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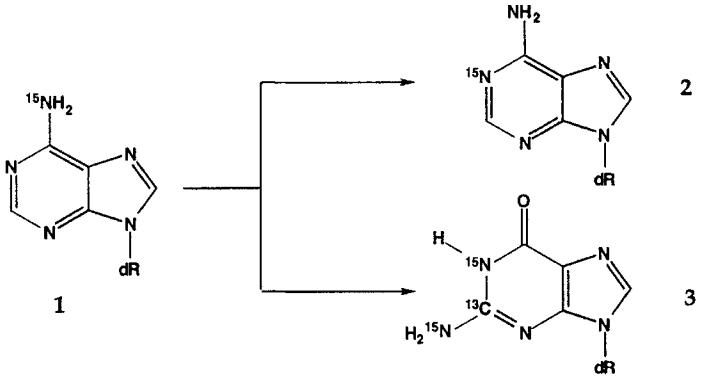
CCC 0362–4803/95/070631–05

Received 11 November 1994
Revised 23 January 1995

Scheme 1



Previous syntheses of both [amino-^{15}N]adenine and 2'-deoxy[amino-^{15}N]adenosine have been reported. For example, 2'-deoxy[amino-^{15}N]adenosine has been constructed in 4 steps from 2'-deoxyadenosine via an intermediate substitution reaction of 2'-deoxy-6-chloropurine and ^{15}N-benzyl amine[6]. [Amino-^{15}N]adenine has been constructed in 3 steps via the addition of ^{15}N-benzyl amine to 6-chloropurine, followed by RuO_4 oxidation and subsequent hydrolysis, in 58% yield[7]. In this synthesis adding to the number of chemical steps is the fact that labeled benzyl amine was constructed in an additional 3 steps from ammonia. [Amino-^{15}N]adenine has also been constructed in a one step process using labeled ammonia and 6-chloropurine in reported 25%[8] and 64%[9] yields. In an effort to increase the efficiency of the synthesis of β-2'-deoxy[amino-^{15}N]adenosine **1** we have revisited the latter procedure and wish to report an improved method, which can be scaled up to give 10 gram quantities, of [amino-^{15}N]adenine in 90-95% yield. Stereospecific conversion of [amino-^{15}N]adenine stereospecifically to β-2'-deoxy[amino-^{15}N]adenosine is accomplished via a coupled *in situ* enzymatic reaction[10].

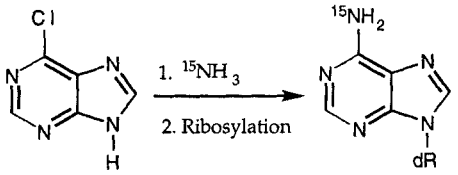
RESULTS AND DISCUSSION

The addition of ^{15}N ammonia to 6-chloropurine **4** was accomplished in a stainless steel Parr 100 mL reaction vessel that was fitted with a pressure gauge and an internal thermocouple. The reaction can either be run using gaseous ammonia (neat), gaseous ammonia and a methanol/water mixture, or with ammonium hydroxide. For example, the reaction was set up by dissolving 10 g of 6-chloropurine in 25 ml of reagent grade methanol and 10 mL of deionized distilled water followed by chilling the Parr vessel to -196°C (evacuated via 3 freeze-thaw cycles to remove any gases present) and subsequently adding 5.0 g of labeled ammonia (4 equiv). The reaction was brought to 120°C (30 psi) for 5 hours. The reaction was monitored using thin layer chromatography (methanol/methylene chloride; 30% v/v) for completion. Upon completion the reaction vessel was cooled and connected to a solution of 1.0 N HCl. The pressure was released and the excess ammonia was then trapped (44% recovery[9]). The resulting solution was concentrated *in vacuo* to give a yellow solid. Examination of the ^{13}C NMR of this material indicated very few impurities. Acidification of the crude adenine with 1.0 N HCl allowed for the isolation of the salt from water/methanol mixtures as a yellow precipitate. Leonard and co-workers[9] have investigated the propensity of adenine to undergo a Dimroth type of rearrangement[11] under autoclaving conditions to give **2** in varying yields. Their results

suggest that these conditions promoted some pyrimidine ring opening between N-1 and C-2 and reclosure to either N-1 or N^6. These authors report that after 48 h (120°C), 24% scrambling was apparent (i.e., 12 % of the ^{15}N label appeared at N-1). In addition, for the rearranged material, they report that H-2 possessed a coupling constant of J = 16 Hz. Based on Leonards report we have detected, upon extended reaction time and temperatures greater than 150 °C, what appears to be a small amount (~2-5%) of the rearranged product (by ^{1}H NMR). Therefore, to suppress this rearrangement, we recommend that reaction times always be less than 12 h.

Conversion to the β-2'-deoxy[amino-^{15}N]adenosine (Scheme 2) **1** was effected *via* the procedure of Jones and co-workers which involves an enzymatic transribosylation reaction using thymidine as the 2'-deoxyribose donor, commercial nucleoside phosphorylase, [amino-^{15}N]adenine·HCl hydrate, and thymidine phosphorylase[12]. The reactions were run at 37°C and are usually complete in 2-3 days. Typically, using 5 g of [amino-^{15}N]adenine·HCl hydrate, the reaction selectively produces β-2'-deoxy[amino-^{15}N]adenosine (generally 5-6 g) **1**.

Scheme 2



4 1

CONCLUSION

This 2-step synthesis of β-2'-deoxy[amino-^{15}N]adenosine **1** from economic precursors is simple and can be conveniently scaled up to provide 5 gram quantities. Quantities of β-2'-deoxy[amino-^{15}N]adenosine **1** can be synthesized in a reasonable time frame (less than 5 days). In addition, purification can be simply accomplished using an anion exchange resin. We are currently exploring common routes to site-specific multiply labeled isotopomers of both the purine and pyrimidine nucleoside families and these will be reported in due course.

Chemicals--[^{15}N]Ammonia (99.2% ^{15}N) was prepared at Los Alamos National Laboratory. 6-Chloropurine was purchased from Aldrich Chemical Co. Thymidine, thymidine phosphorylase, and purine nucleoside phophorylase were purchased from Sigma Chemical Co. **NMR Methods**--Proton, The ^{1}H, ^{13}C, and ^{15}N NMR spectra were recorded as DMSO-d6 or D_2O solutions on a Bruker AM-200, AC-250, WM-300, AMX-500 NMR spectrometers. ^{1}H chemical shifts are expressed in parts per million with respect to tetramethylsilane at 0.0 ppm; ^{13}C chemical shifts are referenced with respect to internal $CDCl_3$ (δ = 77.0 ppm with respect to tetramethylsilane at 0.0 ppm), DMSO (39.5 ppm), CD_3OD (49.0 ppm), or D_2O (external reference doped with methanol); ^{15}N NMR chemical shifts are referenced with respect to 2.5 M solution of potassium [^{15}N]nitrate. Analytical thin-layer chromatography (TLC) was carried out on glass plates (silica gel 60 Å, 250 mm thickness) obtained from EM Scientific. TLC visualization was accomplished with a UV lamp, I_2 staining, and an ethanolic solution of phosphomolybdic acid (PMA).

[Amino-^{15}N]adenine•HCl--Prepared as described above. ^{1}H (DMSO-d6) δ 7.41 (d, J_{1H-15N} = 87 Hz, 2H), 8.11(s, 1H), 8.12 (s, 1H); ^{13}C (DMSO-d6) δ 113.7 (d, $^{5}J_{13C-1H}$ = 9 Hz), 143.7 (d, $^{8}J_{13C-1H}$ = 57 Hz), 145.4 (d, $^{2}J_{13C-1H}$ = 54 Hz), 149.1 (t, C4), 151.4 (d, $^{6}J_{13C-15N}$ = 20 Hz; d, J_{13C-1H} = 20 Hz); ^{15}N (DMSO-d6) δ -276 (t, J_{15N-1H} = 87 Hz; for the free amine J = 71.5 Hz).

β-2'-Deoxy[amino-^{15}N]adenosine--To a 500 mL round bottom flask was added 5 g of [6-^{15}N]adenine hydrochloride hemihydrate (27.5 mmol), 25 g of thymidine (103 mmol), and 400 mL of 10 mM KH_2PO_4 (pH = 7.2). The resulting mixture was stirred for 5 minutes then the pH was adjusted with 1 N KOH to 7.3. Nucleoside phosphorylase (580 units) and thymidine phosphorylase (320 units) were then added. The mixture was brought to 37°C and stirred for 72 h. Purification was accomplished using 500 g of BioRad AG1-X8 (200 mesh) in the hydroxide form. The crude reaction was poured onto the column and a 0-30% methanol/water step gradient was applied. Pooling the appropiate fractions, followed by solvent removal provided 4.69 g of pure material (63% assuming isolation of the hydrate). Further purification can be accomplished using silica gel (230-400 mesh) chromatography and methylene chloride/methanol/ammonium hydroxide (89:10:1) as the eluent. ^{1}H (CD_3OD) δ 2.40 (m, 1H, $H_{2'}$), 2.79 (m, 1H, $H_{2''}$), 3.79 (m, 2H, $H_{5'}$ &$H_{5''}$), 4.07 (q, J = 2.7 Hz, 1H, $H_{4'}$), 4.59 (m, 1H, $H_{3'}$), 6.4 (t, J = 6.8 Hz, 1H, $H_{1'}$), 8.14 (s, 1H, H_2), 8.26 (s, 1H, H_8); ^{13}C (DMSO-d6) δ 40.8 ($C_{2'}$), 63.0 ($C_{5'}$), 72.5 ($C_{3'}$), 86.3 ($C_{1'}$), 89.1 ($C_{4'}$), 120.0 (C_5), 141.2, 149.2, 153.2, 156.5 (d, $^{6}J_{13C-15N}$ = 21 Hz); ^{15}N (DMSO-d6) δ -297.

Acknowledgment. This work was supported by the National Stable Isotopes Resource, NIH Division of Research Resources (RR 02231). We would also like to thank Furman University for the partial support of JK.

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