Preparation, NMR Characterization, and Labeling Reactions of Tritiated Triacetoxy Sodium Borohydride

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Introduction

Reductive hydrogen isotope labeling is still the most desirable method for the incorporation of radioisotopes because of its highly specific nature. Although many hydride reagents are available to synthetic chemists, $NaBH_4$ remains the most widely used reagent in tritium labeling chemistry.[1] The chemistry of $NaBH_4$ is well characterized, and tritiated $NaBH_4$ is commercially available. However, in recent years the biological molecules to be labeled have become more complex and $NaBH_4$ alone cannot offer sufficient selectivity and targeted reactivity. Over the past 45 years, a variety of reagents with a wide range of reactivity and reduction characteristics have been produced by modification of $NaBH_4$.[2,3] For example, for 25 years it has been known that the treatment of $NaBH_4$ with neat acetic acid will modulate its reactivity.[4] Unlike the reaction of $NaBH_4$ with mineral acids or aqueous acids, which leads[5] to diborane formation or complete hydrolysis, the reaction of $NaBH_4$ with neat acetic acid leads to the formation of $NaBH_x(OAc)_y$. $NaBH(OAc)_3$ has many desirable properties because it is a hydride source that is stable in excess acetic acid.[6] The mildness of $NaBH(OAc)_3$ is attributed to both the steric and electron-withdrawing effects of the acetoxy groups, which stabilize the B−H bond.[7] Because of the bulky acetoxy groups $NaBH(OAc)_3$ can be a stereospecific reduction reagent, and it has been used in stereochemically controlled syntheses.[8−10]

An example of the synthetic utility of $NaBH(OAc)_3$ is that it can reduce aldehydes to alcohols in the presence of ketones.[11] It is the reagent of choice over $NaBH_3CN$ in the reductive amination of aldehydes and saturated aliphatic ketones with primary and secondary amines because of its lack of toxicity and toxic byproducts.[12,13] $NaBH(OAc)_3$ was reported to be the preferred reagent over $NaBH_3CN$ for the N-alkylation of amino acid esters by reductive amination.[14,15] It reacts consistently faster, gives better yields, and produces fewer side products.[16] $NaBH(OAc)_3$ was particularly useful in the synthesis of inositol polyphosphates and phosphoinositides by inosose reduction,[17] and the tritiated reagent could be used to produce analogous tritium-labeled compounds.

Results and Discussion

Labeled $NaBH(OAc)_3$ was prepared from $NaBH_4$ and used in simple demonstration reductions as illustrated in Scheme 1. $NaBH_4$ was labeled with either deuterium or tritium by thermal exchange with deuterium or tritium gas, as previously published.[1] The labeled borohydride was then converted to the $NaBH(OAc)_3$ reagent in situ using glacial acetic acid.[4] Once the deuterium- or tritium-labeled $NaBH(OAc)_3$ reagent was prepared, it was used to reduce a simple aldehyde to demonstrate the utility of the reagent.

During the development of a tritium labeling reagent radioactive waste generation may be minimized by the use of deuterium for optimization reactions. The deuterium content of $NaB^2H(OAc)_3$ is not readily determined by direct NMR or mass spectrometric analyses. However, $NaBH(OAc)_3$ reduces 2-naphthaldehyde to 2-naphthalene methanol in high yield (80−90%), and the deuterium content of this product is readily determined by mass spectrometry. Preferred reaction conditions for our syntheses were determined solely on the outcome of % deuterium (%D) in this final reduction product. Initial experiments employed commercial NaB^2H_4 (Sigma Aldrich) to determine the maximum hydrogen isotope content to be expected in the analogous tritium syntheses.

In other work it had been established that benzene is a good solvent for $NaBH(OAc)_3$ formation and that the fourth hydride of $NaBH_4$ could be replaced with acetic acid at 80 °C.[6] Using commercial NaB^2H_4 we determined that benzene was a better dry, aprotic reaction solvent for the formation of $NaB^2H(OAc)_3$ (93%D) than tetrahydrofuran (70%D). In addition, reaction with glacial acetic

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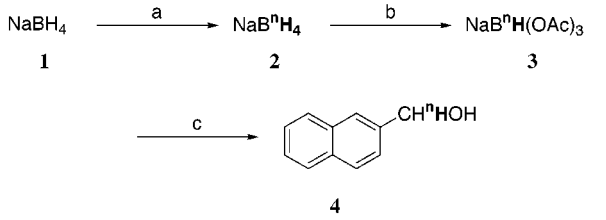
Scheme 1. Summary of $NaBH(OAc)_3$ Synthesis and Naphthaldehyde Reductions with Deuterium ($n = 2$) and Tritium ($n = 3$)[a]



[a] Reaction conditions: (a) $^nH_2$, 450 °C, 6 h; (b) benzene, HOAc, rt, 20 min; (c) naphthaldehyde, THF, 70 °C, 1 h.

acid in benzene at ambient temperature gave higher deuterium incorporation in the product (93%D) than conversion at 85 °C (81%D), confirming the prior studies. In each of these cases the reduction yield of the naphthaldehyde was 80−90%. The deuterium content (93%D) is probably lower in the product as a result of exchange of the borohydride hydrogen atoms under the formation conditions used for the triacetoxy reagent. The ultra-dry, aprotic solvent was chosen to limit the potential for such exchange reactions.

Previous studies have shown that the isotope exchange reaction of $NaBH_4$ with deuterium or tritium gas at elevated temperatures gives isotope incorporation close to 75%.[1] Similar exchange reactions were carried out, and the products were treated with glacial acetic acid in benzene at ambient temperature. To determine the reactivity and selectivity of the product reagents, $NaB^2H(OAc)_3$ and $NaB^3H(OAc)_3$, they were used to reduce 2-naphthaldehyde or a mixture of naphthaldehyde and 2-acetonaphthone. The conversion of 2-naphthaldehyde was found to be as high as 87%, with only a slight reduction (<2%) of 2-acetonaphthone. The isotope incorporation was found to be around 50% in naphthalene methanol, giving a specific activity of 15 Ci/mmol (560 GBq/mmol) for the tritiated product. The lower isotope incorporation may be due to the loss of isotope in the $NaBH(OAc)_3$ by the exchange reaction between acetic acid and the reagent during synthesis.

An aliquot of the tritiated $NaBH(OAc)_3$ reagent was also characterized by 320 MHz ^3H and 96 MHz ^{11}B NMR spectroscopy, and the spectra are shown in Figure 1A−D. Residual NaB^3H_4 peaks (quartet around −0.2 ppm)[1] were barely observable in the ^{11}B coupled ^3H NMR spectrum (Figure 1A). This indicated that the $NaB^3H(OAc)_3$ reagent contained less than 1% residual NaB^3H_4. This is crucial for the selectivity of the $NaB^3H(OAc)_3$ reagent because NaB^3H_4 is more reactive and can reduce other sensitive groups in the target molecules, allowing tritium to enter into unintended positions.[8] The observed ^3H peak for tritiated $NaB^3H(OAc)_3$ at 3.78 ppm was found to be very broad (Figure 1A) as a result of the coupling of ^3H to ^{11}B ($I = 3/2$, 80.42%) and the asymmetric ligand field around the boron. Upon ^{11}B decoupling the broad tritium resonance becomes a sharp single peak with a small shoulder (Figure 1B). The shoulder is most likely due to coupling of ^3H to ^{10}B ($I = 3$, 19.58%) that is not affected by the ^{11}B decoupling.

^{11}B NMR analysis supported the conclusions from the ^3H NMR studies. No peaks belonging to NaB^3H_4 were detected in the ^{11}B NMR spectra of tritiated $NaB^3H(OAc)_3$. The tritium-coupled ^{11}B NMR resonance is a broad multiplet centered at −0.63 ppm (Figure 1C), which is slightly narrowed upon ^3H decoupling (Figure 1D). While the ^3H to ^{11}B coupling is collapsed by the ^3H decoupling, the asymmetric ligand field around the boron remains and ^1H to ^{11}B coupling from the approximately 50% $NaBH(OAc)_3$ present in the reagent solution is still observed.

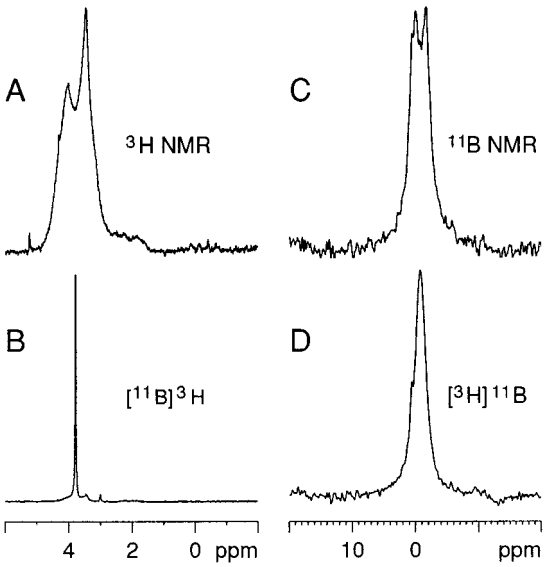


Figure 1. 320 MHz ^3H NMR spectra and 96 MHz ^{11}B NMR spectra of $NaB^3H(OAc)_3$: (A) ^{10}B and ^{11}B coupled ^3H spectrum (6.0 to −2.0 ppm); (B) ^{11}B decoupled ^3H spectrum; (C) ^1H and ^3H coupled ^{11}B NMR spectrum (20 to −20 ppm); and (D) ^3H decoupled ^{11}B spectrum.

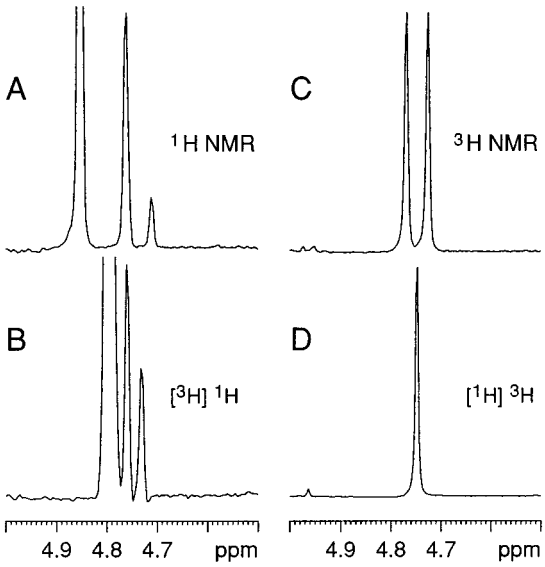
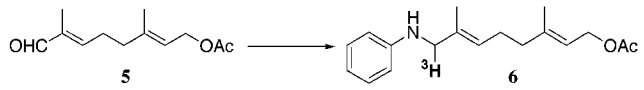


Figure 2. 300 MHz ^1H and 320 MHz ^3H NMR spectra (5.0−4.5 ppm) of the reduction product (in CD_3OD) given by reaction with $NaB^3H(OAc)_3$: (A) ^3H coupled ^1H NMR spectrum; (B) ^3H decoupled ^1H NMR spectrum; (C) ^1H coupled ^3H NMR spectrum; and (D) ^1H decoupled ^3H NMR spectrum.

Tritiated $NaBH(OAc)_3$ was used to reduce a mixture of 2-naphthaldehyde and 2-acetonaphthone, and the 300 MHz ^1H and 320 MHz ^3H NMR spectra of the product are shown in Figure 2A−D. The observed chemical shift, coupling patterns and coupling constants were found to be similar to those previously reported.[1,18] The alcohol product was very clean and the calculated tritium incorporation (using the ratio of R-CH_2-OH, and R-CH^3H-

Scheme 2. Reductive Amination of Aniline with Tritium Incorporation[a]



[a] Reaction conditions: benzene, aniline, $NaB^3H(OAc)_3$, HOAc, $ClCH_2CH_2Cl$, rt, 5 h, dark.

OH in the ^{1}H spectra, R = naphthyl) was 53%, consistent with specific activity estimates from HPLC analyses.

To further demonstrate the utility of tritiated $NaBH(OAc)_3$, we employed it in the multistep synthesis of 8-anilinogeranyl pyrophosphate (AGPP, Scheme 2), which is an alternate substrate for protein farnesyltransferase.[19] The key step in the synthesis of AGPP was formation of allylic amine **6** by reductive amination of aniline with aldehyde **5** and $NaBH(OAc)_3$ in 1,2-dichloroethane. In contrast to the use of $NaBH(OAc)_3$, conventional reductive amination reagents such as $NaBH_3CN/CH_3OH$, BH_3-pyridine, and catalytic hydrogenation gave consistently lower yields of the desired allylic amine with many undesired side products. Reductive amination and incorporation of tritium into **6** was achieved by addition of a mixture of aniline and aldehyde **5** in 1,2-dichloroethane to a slight excess of $NaB^3H(OAc)_3$ in benzene at 25 °C. Tritiated anilino geranyl acetate **6** was isolated in 60% yield with a specific activity of 17 Ci/mmol.

We have previously shown that reductive amination of aniline with **5** using $NaBH(OAc)_3$ in 1,2-dichloroethane results in a mixture of *cis/trans* isomers about the 6,7 double bond in a 7:93 ratio. The 320 MHz ^{3}H NMR spectra of **6** show two proton coupled doublets at 3.68 and 3.59 ppm in a 7:93 ratio, which collapsed into two singlets with ^{1}H decoupling. This indicates that the ratio of *cis/trans* isomers about the 6,7 double bond was not altered by using $NaB^3H(OAc)_3$ or the different solvent system. Acetate **6** was subsequently converted in three steps to 8-[^{3}H]-8-anilinogeranyl pyrophosphate (AGPP), using previously reported chemistry.[19]

Conclusions

Tritiated $NaBH(OAc)_3$ was successfully produced from NaB^3H_4 by reaction with acetic acid. As NaB^3H_4 can be obtained commercially, many tritium labeling chemists could easily adopt this method to prepare $NaB^3H(OAc)_3$ and therefore utilize its characteristic selectivity[20,21] to label complex molecules. We have also demonstrated the possibility of starting from $NaBH_4$, exchange labeling this solid, synthesizing $NaB^3H(OAc)_3$, and reacting it with a substrate, all in a single reaction vessel. Isotope incorporation (both deuterium and tritium) in the final reduction products in our examples were found to be as high as 50% when the reaction started from $NaBH_4$, as described above. Although three of the four hydrides (tritides) of the starting NaB^3H_4 are lost during reagent formation, the mildness and selectivity of $NaB^3H(OAc)_3$ make it the reagent of choice for the incorporation of tritium via reductive amination with α,β-unsaturated aldehydes. This reagent extends the range of reducing agents available to radiochemists to synthesize and incorporate radioisotopes in increasingly complex structures.

Experimental Section

In general, to minimize the generation of radioactive waste, most of the reactions in this study were carried out using deuterium. The actual production of $NaB^3H(OAc)_3$ comprises two parts, viz., tritium gas exchange of $NaBH_4$ and treatment with acetic acid. Initial experiments were carried out using commercial NaB^2H_4 (Sigma Aldrich) to select the most suitable aprotic solvent for reagent synthesis.

Radio HPLC and NMR Spectroscopy. The purity of the deuterated and tritiated $NaBH(OAc)_3$ was determined by ^{1}H (300 MHz), ^{2}H (46 MHz), ^{3}H (320 MHz), and ^{11}B (96 MHz) NMR spectroscopy. All spectra were acquired on a Bruker AF-300 NMR spectrometer. Two 5-mm dual probes were used: tritium/proton and tritium/boron. Deuterium spectra could be obtained using the lock channel of either probe on samples sizes of 500 μL in regular 5 mm NMR tubes. Tritium samples were made to a volume of 300 μL in NMR solvent and doubly encapsulated in Teflon tubes (Wilmad, 507-TR-8″) inside 5-mm screw-cap glass NMR tubes. Spectra were acquired at 298 K, and the referencing of chemical shifts in ^{3}H NMR spectra was achieved by generation of a ghost ^{3}H signal from internal TMS or a solvent signal in the ^{1}H NMR spectrum. Deuterium spectra were referenced to the natural abundance deuterium signal from the solvent. ^{11}B signals from the glass of NMR tubes were minimized by delaying data acquisition until 500 μs after RF excitation, and spectra were referenced to BF_3-$(C_2H_5)_2O$ (δ = 0 ppm) in THF-d_8. Complete details and examples of the NMR procedures are given elsewhere.[1]

The purity of the reduction products was determined by radio HPLC and ^{1}H, ^{2}H, ^{3}H NMR spectroscopy. The specific radioactivity of the tritiated reduction product was determined by liquid scintillation counting of the isolated HPLC peak effluents, as previously described.[1] ^{1}H and ^{3}H NMR were also used to verify the specific radioactivity of the product. All reduction product spectra were acquired on the 5-mm ^{1}H/^{3}H dual probe (deuterium spectra through the lock channel). For deuterated reduction product the %D was determined by mass spectrometry. The detailed procedure is described elsewhere.[1]

Tritium Exchange Reaction of $NaBH_4$. Deuterated and tritiated Na^3BH_4 (typically 0.2 mmol for deuterium experiments and 0.1 mmol for tritium experiments) were produced by hydrogen isotope exchange reactions between $NaBH_4$ and deuterium or tritium gas at elevated temperatures. Details of the method are described elsewhere.[1] This method usually gives an isotope incorporation of around 75% in $NaBH_4$.

Sodium Triacetoxyborotritide and -borodeuteride. After the exchange reaction, the vessel was cooled to room temperature, excess tritium or deuterium gas was removed, and the sample vessel was flushed twice with dry N_2. Nitrogen gas was again admitted to a pressure of 80 kPa, and benzene (300 μL) was injected. A magnetic stirrer was moved into the lower part of the vessel, and the sample was stirred. The required amount of glacial acetic acid (typically, 0.245 mmol, 3.5-fold molar excess of $NaBH_4$) was then injected while the sample was under constant stirring. A white fluffy solid of $NaBH(OAc)_3$ formed immediately, and the pressure inside the vessel rose as a result of the evolution of hydrogen (deuterium or tritium) gas. After the sample stirred for 20 min at ambient temperature, the hydrogen gas, the solvent, and the excess acetic acid were all removed by evacuation. The vessel was then flushed three times with dry nitrogen and filled with nitrogen to a pressure of 80 kPa. Dry deuterated dimethyl sulfoxide (DMSO-d_6, 500 μL) was injected and the sample stirred. An aliquot (100 μL) of the sample solution was then removed for characterization by ^{3}H and ^{11}B NMR techniques. The subsequent reduction reaction

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with this batch of reagent was done in a mixed DMSO/tetrahydrofuran (THF) solvent system. Deuterium experiment: ^{11}B selectively decoupled ^{2}H NMR (DMSO) δ 3.72 ppm; ^{2}H selectively decoupled ^{11}B NMR (DMSO-d_6), unreferenced. Tritium experiment: ^{11}B decoupled ^{3}H NMR (DMSO-d_6) δ 3.78; ^{3}H decoupled ^{11}B NMR (DMSO-d_6) δ −0.63.

Reduction of Naphthaldehyde and Naphthaldehyde/Acetonaphthone Mixture. After the production of deuterated or tritiated $NaBH(OAc)_3$ the vessel was flushed three times with dry nitrogen and filled with nitrogen to the pressure of 80 kPa. Dry THF (300 μL) was injected, and the sample was stirred. A solution of 2-naphthaldehyde in THF (300 μL) was injected into the vessel. The molar ratio of substrate and reagent was kept at 4.0. For the selectivity experiment, a mixture of 2-naphthaldehyde and 2-acetonaphthone (0.012 mmol of each substrate in 300 μL of THF) was injected. The pressure inside the vessel was maintained at 90 kPa. After 1 h of stirring at 70 °C the reaction vessel was cooled to ambient temperature, and the excess reagent quenched with 500 μL of methanol. The solvent was removed by evacuation. The quenching process was repeated, and the vessel was then filled with dry N_2 and detached from the vacuum line. The reaction product was then dissolved in ether (2.5 mL), and the borate salts were separated by washing with water (1 mL, 3×). Ether was removed by lyophilization, and the residual solids were dissolved in CD_3OD for liquid scintillation counting, radio-HPLC, and ^{1}H and ^{2}H or ^{3}H NMR analyses. For deuterated products the sample was also analyzed by mass spectrometry. Details of the methods are described elsewhere.[1,18,22] Deuterium experiment: reduction yield 83%; 50% ^{2}H incorporation; MS *m*/*z* 160 (6.1), 159 (50.7), 158 (43.2); ^{1}H NMR (CD_3OD) δ 4.76 (CH_2−OH), 4.74 (CH^2H−OH). Tritium experiment: reduction yield 87%; 53% ^{3}H incorporation [15.3 Ci/mmol (560 GBq/mmol)] by ^{1}H NMR (CD_3OD) δ 4.76 (CH_2−OH 64 mol %), δ 4.73 (CH^3H−OH 36 mol %), ($\Delta\delta_T = 0.029 \pm 0.001$ ppm); ^{3}H NMR (CD_3OD), δ 4.77, 4.73 (CH^3H−OH), J_{H-T} = 13.68 Hz; ^{1}H decoupled ^{3}H NMR (CD_3OD), δ 4.75 (CH^3H−OH).

Reductive Tritioamination of (*E*,*E*)-3,7-Dimethyl-1-acetoxy-2,6-octadien-8-al. A description of the analogous nonradioactive synthesis has recently been published.[19] For this reaction, $NaB^3H(OAc)_3$ was produced by the exchange labeling of NaB^2H_4 (3.5 mg, 0.1 mmol) in the presence of tritium gas and derivatizing as described above. The resultant solution was dried under vacuum, and the hydride was redissolved in 500 μL of benzene. All reactions and handling after this step were conducted under red light to limit photodegradation of the products. To the benzene solution of hydride was added a mixture of dimethyl-1-acetoxy-2,6-octadien-8-al (**5**, 16 μL), dry glacial acetic acid (4 μL), and aniline (6 μL) in 1,2-dichloroethane (1 mL). After the mixture stirred at ambient temperature for 5 h, the reaction was quenched with 5% $NaHCO_3$ (500 μL), dried under vacuum, and extracted with diethyl ether. The ether layer was dried through $MgSO_4$, and the ether removed using a stream of nitrogen gas. The residue was dissolved in CD_3OD for characterization by liquid scintillation counting, NMR spectroscopy, and HPLC. Radioactive yield approximately 1 Ci; calculated specific activity from HPLC analysis = 17 Ci/mmol (610 GBq/mmol); ^{1}H NMR (CD_3OD) δ 7.05 (t, 2H), 6.56 (m, 3H) 5.38 (m, 1H), 5.29 (m, 1H), 4.54 (d, 2H), 3.64 (m, 1H), 2.18−2.03 (m, 4H), 2.00 (s, 3H), 1.69 (s, 3H), 1.64 (s, 3H); ^{3}H NMR (CD_3OD), δ 3.68 (d, 7%), J_{H-T} = 14.52 Hz, 3.59 (d, 93%), J_{H-T} = 16.96 Hz; ^{1}H decoupled ^{3}H NMR (CD_3OD), δ 3.68 (s, 7%), 3.59 (s, 93%).

The complete synthesis of 8-[^{3}H]-8-anilinogeranyl pyrophosphate (AGPP) involved elaboration through three further reactions starting with this radioactive product, using chemistry similar to that published for the nonradioactive material.[19] The crude product from the reductive amination reaction was purified by flash chromatography, and fractions were collected. The fractions were analyzed by UV spectroscopy, and several fractions were combined to yield 730 mCi of tritiated material with the appropriate UV characteristics. Approximately 320 mCi of this purified acetate **6** was converted to the alcohol by base hydrolysis, the product solution was neutralized, and the product was extracted and dried. Liquid scintillation counting of the alcohol product gave 318 mCi, which was all carried forward to the chlorination step, after which the product was again purified by flash chromatography. Pyrophosphorylation and column purification according to the published procedure[19] yielded 20 mCi of the desired AGPP, which was further analyzed and purified by HPLC to yield 10 mCi of the final product. ^{1}H NMR (D_2O) δ 7.26 (t, 2H), 6.82 (m, 3H), 5.42 (m, 2H), 4.47 (m, 2H), 3.71 (m, 0.07H), 3.65 (m, 0.93H), 2.16−2.08 (m, 4H), 1.69 (s, 3H), 1.62 (s, 3H); ^{3}H NMR (D_2O), δ 3.67, 3.62, J_{H-T} = 15.53 Hz; ^{1}H decoupled ^{3}H NMR (D_2O), δ 3.68.

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Supporting Information Available: ^{1}H NMR spectrum for 8-anilinogeranyl pyrophosphate. ^{1}H, ^{3}H, and [^{1}H]^{3}H NMR spectra for 8-[^{3}H]-8-anilinogeranyl pyrophosphate. This material is available free of charge via the Internet at http://pubs.acs.org.

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