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A Direct, Efficient Method for the Preparation of N^6-Protected ^{15}N-Labeled Adenosines

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Abstract: N^6-Protected adenosines have been prepared from inosines by activation of the C6 position and Pd-catalyzed coupling with amides. An efficient route to [6-$^{15}NH_2$]-*N*^{6}-benzoyladenosine and [1-^{15}N,6-$^{15}NH_2$]-*N*^{6}-benzoyladenosine has been achieved.

Specific ^{15}N labeling of nitrogen atoms of nucleosides and nucleotides has become a very useful tool for obtaining key information on the local interactions involved in molecular recognition processes.[1] In this connection, NMR of ^{15}N-labeled nucleobases[2] has provided direct evidence of hydrogen bonding, protonation, hydration, or ligand interactions.[2b]

The synthesis of oligonucleotides with ^{15}N at relevant sites has been achieved by two different tactics: (i) introduction of the exocyclic ^{15}N labels in the deprotection step of a modified oligonucleotide[3] and (ii) preparation of labeled nucleosides followed by their incorporation into DNA or RNA oligomers.[2a] The second approach has prompted the chemical synthesis of numerous ^{15}N-labeled nucleosides.[2a,4]

Selective labeling of the amino group of adenosine has been a well-established target. The most common approach has involved the reaction of C6-activated purine derivatives[5] (usually 6-chloropurines)[5a−i] with an excess of $^{15}NH_3$. To avoid the expenses of labeling reagent, stoichiometric amounts of [^{15}N]benzylamine and other ammonia equivalents have replaced $^{15}NH_3$ as the ^{15}N source.[6] As a result, the key intermediate to be incorporated into an oligonucleotide chain is a nucleoside that has the amino group either benzylated or unprotected. However, in oligonucleotide synthesis, amides are the most frequent protecting groups for that position. They are stable and unreactive during the elongation process and can be removed in the final step under ammonolysis conditions.[7]

Herein, we describe a simple method for label introduction by direct stoichiometric addition of ^{15}N-labeled amides on purines activated at position 6. We decided to explore the Pd- and Cu-catalyzed methodologies for the formation of C−N bonds.[8] Despite the very recent and significant applications of these reactions on nucleosides,[9] amides have not been used yet as coupling species[10] on either purines or pyrimidines.

Our first attempt of coupling benzamide with 6-bromopurine **1a** (Table 1) was very promising. The addition product **2a** was obtained in 45% yield by using Pd_2dba_3 and BINAP as the catalytic system and Cs_2CO_3 as a base, in toluene at 80 °C. However, a significant amount of byproducts was obtained, including a disubstituted product ("dimer").[11] The formation of byproducts was not

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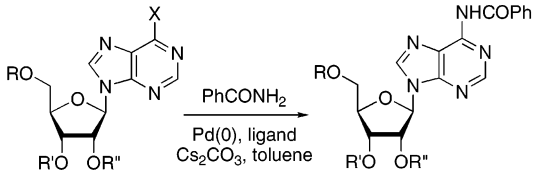
TABLE 1. Addition of Benzamide to 6-Halopurines[a]



1a (R = R' = R" = Ac, X = Br) **2a** (R = R' = R" = Ac)
1a' (R = R' = R" = Ac, X = Cl) **2a** (R = R' = R" = Ac)
1b (R = TBS, R'R" = CMe_2, X = Br) **2b** (R = TBS, R'R" = CMe_2)

entry	compd	ligand	product	yield[b,c] (%)
1	**1a**	BINAP	**2a**	45
2	**1a**	dppf	**2a**	74 (81)
3	**1b**	dppf	**2b**	84 (84)
4	**1a′**	dppf	**2a**	52

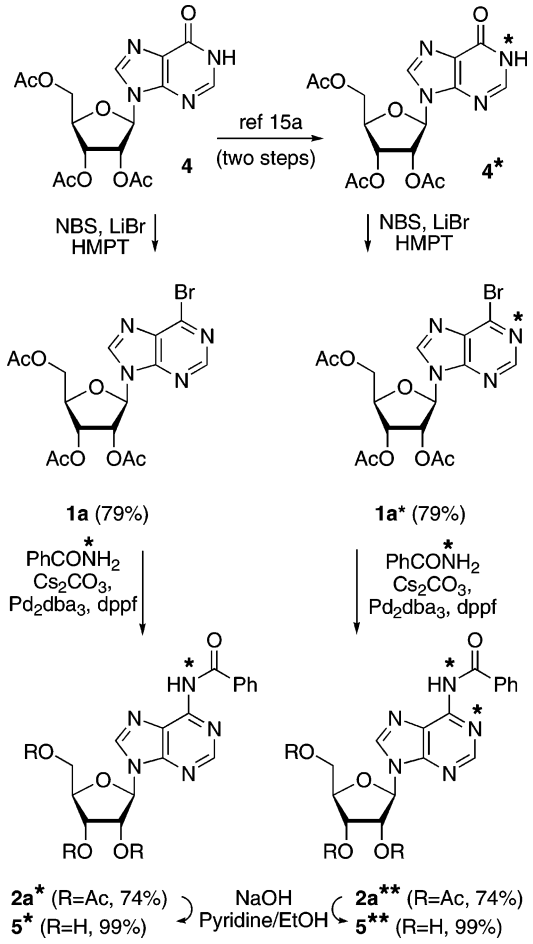
[a] The reactions were performed with $Pd_2(dba)_3$·$CHCl_3$ (5 mol %), ligand (15 mol %), Cs_2CO_3 (1.4 equiv), and benzamide (1.1 equiv) in toluene at 80 °C. [b] Isolated yield. [c] Within parentheses, yield based on recovered starting material (brsm).

avoided when other solvents, temperatures, ligands, and bases were used. Furthermore, the reaction did not work in the absence of catalyst, as starting material was mostly recovered. Only when 1,1′-bis(diphenylphosphino)ferrocene (dppf) was used as a ligand, yields increased (74%) and the "dimer" formation was prevented (entry 2 in Table 1). Functional groups other than esters, such as *tert*-butyldimethylsilyl ethers or isopropylidene acetals (see **1b**), were also compatible with the reaction conditions; in fact, nucleoside **1b** reacted with benzamide to afford the addition product **2b** in 84% yield (entry 3). As expected, chloropurines turned out to be less reactive, as yields were lower when the reaction was performed with nucleoside **1a′** (entry 4).[12]

The optimized conditions (entry 2 and 3) can be applied to the synthesis of 2′-deoxyadenosines, since the Pd-catalyzed addition of benzamide to 6-bromo-9-(3′,5′-di-*O*-acetyl-2′-deoxy-β-D-ribofuranosyl)-9*H*-purine (**1c**) afforded 3′,5′-di-*O*-acetyl-N^6-benzoyl-2′-deoxyadenosine (**2c**), also in good yield (66%, 79% brsm). Furthermore, amides other than benzamide also react, as expected. For example, acetamide and **1a** afforded $N,^6O^{2'},O^{3'},O^{5'}$-tetraacetyladenosine (**3a**) in good yield (60%, 86% brsm) when treated with Pd(0) and dppf (as in entry 2).

Since adenosines and 2′-deoxyadenosines are incorporated very often in oligonucleotide chains with the amino group protected as a benzamide, the addition of labeled benzamide to bromopurine **1a** seemed the more straightforward approach to the synthesis of N6-labeled adenosines from inosines.

SCHEME 1. Synthesis of [6-$^{15}NH_2$]-N^6-Benzoyladenosine (5*) and [1-^{15}N,6-$^{15}NH_2$]-N^6-Benzoyladenosine (5)**



Thus, protected inosine **4** was converted into bromopurine **1a** in 79% yield by reaction with NBS in the presence of HMPT (hexamethylphosphorous triamide, $(Me_2N)_3P$) and LiBr[13] (Scheme 1). Coupling of **1a** with labeled benzamide[14] was performed under the optimized conditions shown in Table 1 (entry 2) to afford monolabeled adenosine **2a*** in 74% yield (81% brsm). Deprotection of acetyl groups was easily achieved in 99% yield by basic hydrolysis (NaOH/pyridine/EtOH). The ^{15}N NMR spectrum of deprotected monolabeled *N*-benzoyladenosine **5*** showed a doublet at δ −230.8 ($^1J_{NH}$ = 85 Hz), and the ^{13}C NMR spectrum showed the expected splitting on the purine ring (C6, d, $^1J_{CN}$ = 18.4 Hz).

The same procedure can be used to prepare double-labeled adenosine **5****. Protected inosine **4** was converted into protected [1-^{15}N]inosine **4*** in 63% yield by using our two-step procedure[15] (*N*-nitration, ANRORC process with $^{15}NH_3$) that employs only 1 equiv of $^{15}NH_4Cl$ as the source of ^{15}N (Scheme 1). Activation of the C6 position was

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(11) Two units of nucleoside are linked to the benzamide nitrogen atom.

(12) In this case, we also checked Cu-catalyzed couplings with *trans*-1,2-cyclohexanediamine and CuI (ref 10e) but the yields were even lower.

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(14) [^{15}N]Benzamide can be prepared quantitatively from $^{15}NH_4Cl$ and benzoyl chloride (see ref 15a).

performed by reaction of **4*** with NBS in the presence of HMPT and LiBr.[13] Pd-catalyzed addition of 1.1 equiv of labeled benzamide[14] to the labeled 6-bromopurine **1a*** afforded the double-labeled adenosine **2a**** in 74% yield which was deacetylated to the deprotected double-labeled *N*-benzoyladenosine **5****. The spectroscopic data of **5**** showed a doublet at δ −101.9 ($^2J_{NH}$ = 15 Hz) and a doublet at δ −230.8 ($^1J_{NH}$ = 85 Hz) in the proton-coupled ^{15}N NMR spectrum;[16] the splitting of C6 (dd, $^1J_{CN}$ = 18.4 Hz, $^1J_{CN}$ = 4.6 Hz) in the ^{13}C NMR spectrum confirmed the incorporation of the second label.

In conclusion, we have developed a simple procedure for the introduction of amides in position 6 of purines. The overall process can be used to prepare directly ^{15}N-labeled adenosines protected as amides.

Experimental Section

For general methods, see ref 15. Compound **1a′** was prepared according to ref 17. Coupling constants (*J*) are given in Hz. ^{15}N NMR chemical shifts are referred to external concentrated H^{15}NO$_3$ (negative values upfield). HRMS were registered in the FAB positive mode.

[1-^{15}N]-6-Bromo-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (1a*). To a solution of [1-^{15}N]-2′,3′,5′-tri-*O*-acetylinosine[15] (**4***, 125 mg, 0.32 mmol) and *N*-bromosuccinimide (169 mg, 0.95 mmol) in CH_3CN (5 mL) at −20 °C was added dropwise hexamethylphosphorous triamide (HMPT, $(Me_2N)_3P$) (144 μL, 0.79 mmol). The reaction mixture was stirred at room temperature for 0.5 h. Afterward, LiBr (137 mg, 1.58 mmol) was added, and the reaction mixture was heated at 70 °C for 5 h. The brown mixture was cooled to room temperature, and the volatile materials were removed by rotatory evaporation. Purification of the crude product by flash chromatography (CH_2Cl_2−MeOH 98:2) afforded 114 mg (79%) of [1-^{15}N]-6-bromo-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**1a***) as a pale yellow foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.73 (d, *J* = 15.9 Hz, 1H), 8.32 (s, 1H), 6.23 (d, *J* = 5.1 Hz, 1H), 5.96 (dd, *J* = 5.2, 5.1 Hz, 1H), 5.65 (dd, *J* = 5.2, 4.4 Hz, 1H), 4.50−4.37 (m, 3H), 2.16 (s, 3H), 2.12 (s, 3H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.2, 169.5, 169.3, 152.2 (d, *J* = 4.6 Hz), 149.9 (d, *J* = 2.7 Hz), 143.7 (d, *J* = 2.1 Hz), 143.4, 134.9 (d, *J* = 2.7 Hz), 86.9, 80.5, 73.1, 70.4, 62.8, 20.7, 20.5, 20.3; ^{15}N NMR ($CDCl_3$, 30 MHz) δ −61.1 (d, *J* = 16 Hz); HRMS (FAB) calcd for $C_{16}H_{18}BrN_3{}^{15}NO_7$ (M + H)$^+$ 458.0329, 460.0309, found 458.0325, 460.0319.

General Procedure for the Reaction of 6-Bromopurine Nucleosides with Benzamide. An oven-dried vial was charged with Pd_2dba_3·$CHCl_3$ (0.05 mmol, 10 mol % Pd) and 1,1′-bis-(diphenylphosphino)ferrocene (dppf, 0.15 mmol, 1.5 equiv/Pd). The vial was capped with a rubber septum, and a solution of nucleoside (1.00 mmol) in toluene (3.0 mL) was added via cannula. Then, benzamide (1.10 mmol) and Cs_2CO_3 (1.40 mmol) were added as solids, and the resulting yellow mixture was stirred at 80 °C. The reaction was monitored by thin-layer chromatography. After complete consumption of the starting nucleoside, the resulting brown suspension was allowed to cool to room temperature and concentrated in vacuo. The crude material was adsorbed onto silica gel and purified by flash chromatography (hexanes−EtOAc 25:75 and later CH_2Cl_2−MeOH from 99:1 to 98:2).

[6-^{15}NH$_2$]-2′,3′,5′-Tri-*O*-acetyl-*N*^{6}-benzoyladenosine (2a*). The above general procedure was followed using 6-bromo-9-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine[13] (**1a**, 55 mg, 0.12 mmol) and [^{15}N]-benzamide.[14] After 3 h at 80 °C, the purification of the crude product by flash chromatography gave 5 mg (9%) of starting material **1a** and 44 mg (74%) of [6-^{15}NH$_2$]-2′,3′,5′-tri-*O*-acetyl-*N*^{6}-benzoyladenosine (**2a***) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.08 (d, *J* = 88.4 Hz, 1H), 8.80 (s, 1H), 8.19 (s, 1H), 8.03 (d, *J* = 7.2 Hz, 2H), 7.63−7.51 (m, 3H), 6.27 (d, *J* = 5.5 Hz, 1H), 5.96 (t, *J* = 5.5 Hz, 1H), 5.68 (dd, *J* = 5.5, 4.3 Hz, 1H), 4.50−4.38 (m, 3H), 2.16 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 169.5, 169.3, 164.6 (d, *J* = 13.0 Hz), 152.9 (d, *J* = 2.3 Hz), 151.7, 149.7 (d, *J* = 19.9 Hz), 141.2, 133.4 (d, *J* = 9.2 Hz), 132.8, 128.8, 127.9, 123.6 (d, *J* = 2.3 Hz), 86.3, 80.5, 73.1, 70.6, 63.0, 20.7, 20.5, 20.3; ^{15}N NMR ($CDCl_3$, 30 MHz) δ −236.2 (d, *J* = 88 Hz); HRMS (FAB) calcd for $C_{23}H_{24}N_4{}^{15}NO_8$ (M + H)$^+$ 499.1595, found 499.1579.

[1-^{15}N,6-^{15}NH$_2$]-2′,3′,5′-Tri-*O*-acetyl-*N*^{6}-benzoyladenosine (2a).** The above general procedure was followed using [1-^{15}N]-6-bromo-(2′,3′,5′-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**1a***, 56 mg, 0.122 mmol) and [^{15}N]benzamide.[14] After 3 h at 80 °C, the purification of the crude product by flash chromatography gave 45 mg (74%) of [1-^{15}N,6-^{15}NH$_2$]-2′,3′,5′-tri-*O*-acetyl-*N*^{6}-benzoyladenosine (**2a****) as a colorless oil: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 9.09 (dd, *J* = 88.8, 2.0 Hz, 1H), 8.80 (d, *J* = 15.8 Hz, 1H), 8.19 (s, 1H), 8.03 (d, *J* = 7.2 Hz, 2H), 7.63−7.51 (m, 3H), 6.26 (d, *J* = 5.5 Hz, 1H), 5.96 (t, *J* = 5.5 Hz, 1H), 5.68 (dd, *J* = 5.5, 4.3 Hz, 1H), 4.49−4.37 (m, 3H), 2.17 (s, 3H), 2.13 (s, 3H), 2.09 (s, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.3, 169.5, 169.3, 164.6 (d, *J* = 13.0 Hz), 152.9 (dd, *J* = 2.7, 2.3 Hz), 151.7 (dd, *J* = 2.3, 1.2 Hz), 149.7 (dd, *J* = 19.9, 5.2 Hz), 141.2, 133.4 (d, *J* = 9.2 Hz), 132.8, 128.8, 127.9, 123.6 (dd, *J* = 3.1, 2.3 Hz), 86.3, 80.5, 73.1, 70.6, 63.0, 20.7, 20.5, 20.3; ^{15}N NMR ($CDCl_3$, 30 MHz) δ −236.1 (dd, *J* = 89, 4 Hz), −114.0 (dd, *J* = 16, 4 Hz); HRMS (FAB) calcd for $C_{23}H_{24}N_3{}^{15}N_2O_8$ (M + H)$^+$ 500.1566, found 500.1556.

[1-^{15}N,6-^{15}NH$_2$]-*N*^{6}-Benzoyladenosine (5).** To a solution of [1-^{15}N,6-^{15}NH$_2$]-2′,3′,5′-tri-*O*-acetyl-*N*^{6}-benzoyladenosine (**2a****, 36 mg, 0.072 mmol) in ethanol−pyridine 1:1 (v/v, 439 μL), a mixture of 2 N NaOH and ethanol (299 μL + 299 μL) was added. After the mixture was stirred for 6 min at room temperature, Amberlite IR-120 was added to neutralize the base. The resin was filtered and washed with ethanol (1 mL) and pyridine (1 mL). Combined filtrates were evaporated, and the crude product was purified by flash chromatography (CH_2Cl_2−MeOH 80:20) to give 27 mg (99%) of [1-^{15}N,6-^{15}NH$_2$]-*N*^{6}-benzoyladenosine (**5****): ^{1}H NMR (DMSO-d_6, 400 MHz) δ 11.17 (d, *J* = 84.6 Hz, 1H), 8.76 (d, *J* = 15.4 Hz, 1H), 8.72 (s, 1H), 8.05 (d, *J* = 7.6 Hz, 2H), 7.68−7.53 (m, 3H), 6.05 (d, *J* = 6.0 Hz, 1H), 5.55 (d, *J* = 5.7 Hz, 1H), 5.25 (d, *J* = 3.9 Hz, 1H), 5.13 (t, *J* = 5.7 Hz, 1H), 4.66 (m, 1H), 4.20 (m, 1H), 3.99 (m, 1H), 3.74−3.55 (m, 2H); ^{13}C NMR (DMSO-d_6, 100 MHz) δ 165.7 (d, *J* = 13.0 Hz), 152.2 (dd, *J* = 2.7, 1.2 Hz), 151.6, 150.4 (dd, *J* = 18.4, 4.6 Hz), 143.1, 133.4 (d, *J* = 9.2 Hz), 132.4, 128.5, 125.9 (dd, *J* = 3.1, 1.5 Hz), 87.6, 85.7, 73.7, 70.4, 61.3; ^{15}N NMR (DMSO-d_6, 30 MHz) δ −230.8 (broad d, *J* = 85 Hz),[16] −101.9 (broad d, *J* = 15 Hz); HRMS (FAB) calcd for $C_{17}H_{17}N_3{}^{15}N_2NaO_5$ (M + Na)$^+$ 396.1068, found 396.1065.

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Supporting Information Available: Experimental procedures and characterization data of compounds **2b**, **2c**, **3a**, and **5***. ^{1}H, ^{13}C and ^{15}N NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) The key intermediate is 2′,3′,5′-triacetyl-1-nitroinosine: (a) Ariza, X.; Bou, V.; Vilarrasa, J. *J. Am. Chem. Soc.* **1995**, *117*, 3665−3673. For the use of *N*-nitronucleobases in ^{15}N-labeling, see also: (b) Ariza, X.; Bou, V.; Vilarrasa, J.; Tereshko, V.; Campos, J. L. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2454−2455. (c) Ariza, X.; Farràs, J.; Serra, C.; Vilarrasa, J. *J. Org. Chem.* **1997**, *62*, 1547−1549. (d) Ariza, X.; Vilarrasa, J. *J. Org. Chem.* **2000**, *65*, 2827−2829.

(16) This last splitting was only observed in dilute samples. In concentrated samples, fast proton exchanges prevented to see this splitting.

(17) Zemlicka, J.; Owens, J. *Nucl. Acid Chem.* **1978**, *2*, 611−614.