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# [N,1-$^{15}N_2$]-2′-Deoxyadenosines

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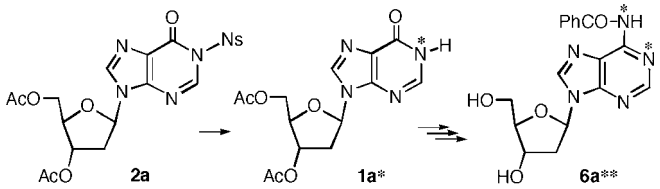
## ABSTRACT

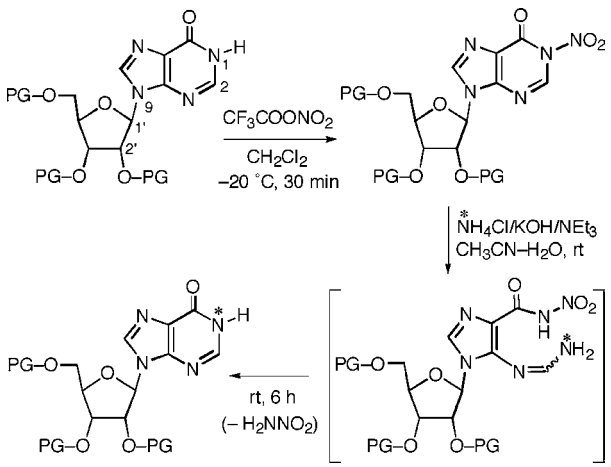



Ns = 2-nitrobenzenesulfonyl

**An efficient route to deoxyadenosine derivatives labeled on both the amino group and nitrogen 1 is uncovered. First, 3′,5′-di-*O*-acetyl-2′-deoxy-1-(2-nitrobenzenesulfonyl)inosine (2a) and only 1.1 equiv of $^{15}NH_4Cl$ are used for labeling position 1 (1a*) through the isolation of the open intermediate and its cyclization with DBU in anhydrous $CH_3CN$. Inosine 1a* is then converted to [N,1-$^{15}N_2$]-3′,5′-di-*O*-acetyl-*N*$^6$-benzoyl-2′-deoxyadenosine (5a**, the precursor of 6a**) via a Pd/dppf-catalyzed chloride-to-benzamide replacement, by using again only 1.1 equiv of the labeling source.**

Purine nucleosides specifically labeled on the nitrogen atoms involved in Watson−Crick nucleobase pairings[1] are enjoying an increasing number of applications.[2] Activation of N1 of inosine derivatives via N-nitration, followed by reaction with $^{15}N$-labeled ammonia (see Scheme 1, where N* means 98% of $^{15}N$), was shown to be an excellent method for the labeling of that position via an ANRORC-like mechanism.[3,4] In principle, it could be expected that the stronger the electron-withdrawing group (EWG) on N1, the higher the reactivity of the starting material with nitrogen nucleophiles.

**Scheme 1**




However, application of our standard procedure (activation by N-nitration)[3] to the 2′-deoxyinosine series (**1a**−**c**, Scheme

(1) For reviews, see: (a) Kawashima, E.; Kamaike, K. *Mini-Rev. Org. Chem.* **2004**, *1*, 309. (b) Lagoja, I. M.; Herdewijn, P. *Synthesis* **2002**, 301. (c) Milecki, J. *J. Labelled Compd. Radiopharm.* **2002**, *45*, 307. Also see: (d) Shallop, A. J.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem.* **2003**, *68*, 8657 and references therein. (e) Abad, J.-L.; Gaffney, B. L.; Jones, R. A. *J. Org. Chem.* **1999**, *64*, 6575 and references therein.

(2) For recent representative examples, see: (a) Wenter, P.; Pitsch, S. *Helv. Chim. Acta* **2003**, *86*, 3955. (b) Gorchs, O.; Hernández, M.; Garriga, L.; Pedroso, E.; Grandas, A.; J. Farràs, J. *Org. Lett.* **2002**, *4*, 1827. (c) Dunger, A.; Limbach, H.-H.; Weisz, K. *J. Am. Chem. Soc.* **2000**, *122*, 10109. (d) Matsuo, H.; Moriguchi, T.; Takagi, T.; Kusakabe, T.; Buratowski, S.; Sekine, M.; Kyogoku, Y.; Wagner, G. *J. Am. Chem. Soc.* **2000**, *122*, 2417.

(3) (a) Ariza, X.; Bou, V.; Vilarrasa, J. *J. Am. Chem. Soc.* **1995**, *117*, 3665. (b) Terrazas, M.; Ariza, X.; Farràs, J.; Guisado-Yang, J. M.; Vilarrasa, J. *J. Org.* Chem. **2004**, *69*, 5473 and references therein.

(4) For the use of 4-nitrophenyl and 2,4-dinitrophenyl derivatives in the deoxyinosine series, see: (a) De Napoli, L.; Messere, A.; Montesarchio, D.; Piccialli, G. *J. Org. Chem.* **1995**, *60*, 2251. (b) Catalanotti, B.; De Napoli, L.; Galeone, A.; Mayol, L.; Oliviero, G.; Piccialli, G.; Varra, M. *Eur. J. Org. Chem.* **1999**, 2235 and references therein.

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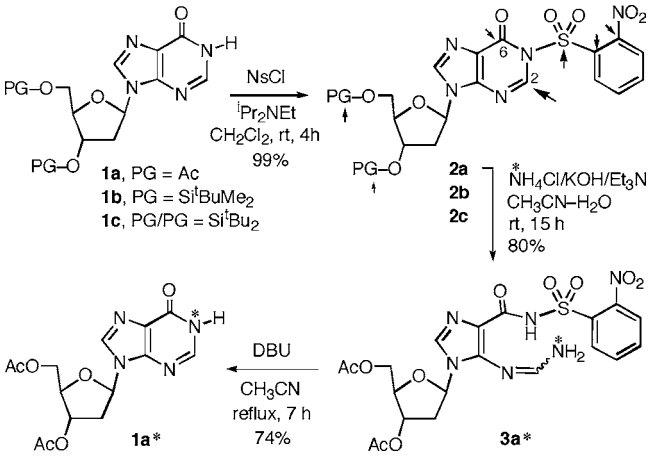
2) was unsuccessful due to the instability of these deoxynucleosides in the nitration medium, where $CF_3COONO_2$ is generated from $NH_4NO_3/(CF_3CO)_2O$. The highest yield of the N-nitro derivative of **1a** was 20%; moreover, the product decomposed during the workup. Other nitrating agents were also unsatisfactory.[5] It is reported here how we have overcome this problem. This has allowed us to synthesize [N,1-$^{15}N_2$]-2′-deoxyadenosines, also called [1,$NH_2$-$^{15}N_2$]-2′-deoxyadenosines, which are described for the first time, to the best of our knowledge.

**Scheme 2**




Reaction of deoxyinosines **1a**−**c** with a set of $ArSO_2Cl$ and $^iPr_2NEt$ in $CH_2Cl_2$ at room temperature afforded the desired $N^1$-sulfonyl derivatives in 95−100% yields. Many of them reacted very slowly with 1.1 equiv of $^{15}NH_3$, while those containing a strong EWG (e.g., triflyl, 2,4-dinitrobenzenesulfonyl, and 4-nitrobenzenesulfonyl) gave rise to complex mixtures arising from desulfonylation and/or $S_NAr$ reactions (to be reported elsewhere). The solution relied on the use of the 2-nitrobenzenesulfonyl group (the *o*-nosyl group, Ns); see **2a**−**c**, Scheme 2.[6,7]

In fact, with di-*O*-acetyl derivative **2a**, $^{15}NH_3$ attacks mainly (95%) at the desired C2 position and in a small percentage (5%) at S. We utilized 1.10 equiv of $^{15}NH_4Cl$, 1.05 equiv of KOH, and 1.05 equiv of $Et_3N$, in a 0.1 M solution of **2a** in 3:1 v/v $CH_3CN-H_2O$ (or in 8:1 1,4-dioxane−$H_2O$ with almost the same result, viz. 90% attack at C2 and 10% at S). Attacks at other possible positions ($C_{ipso}$ to $SO_2$, $C_{ortho}$, acetyl groups, C6), pointed out with small arrows in Scheme 2, were not detected in this case. The direct desulfonylation reaction, giving starting material **1a** and [$^{15}$N]-nosylamide, and the indirect desulfonylation that may arise from the $C_{ipso}$ attack (to afford **1a**, $SO_2$, and [$^{15}$N]-2-nitrobenzenamine) had to be avoided as much as possible, since the final labeled product **1a*** would be otherwise contaminated with high percentages of unlabeled **1a** coming from these secondary pathways. The presence of a nitro group in close vicinity to the sulfonyl group is apparently sufficient to hinder the attack at these two positions.

The crude product containing intermediate **3a*** did not cyclize spontaneously to **1a***. In other words, at room temperature, intermediate **3a*** was kinetically stable. Unfortunately, on heating in the same medium, without additives or with addition of either acid or base, only hydrolysis products of **3a*** were obtained (and no products of cyclization).

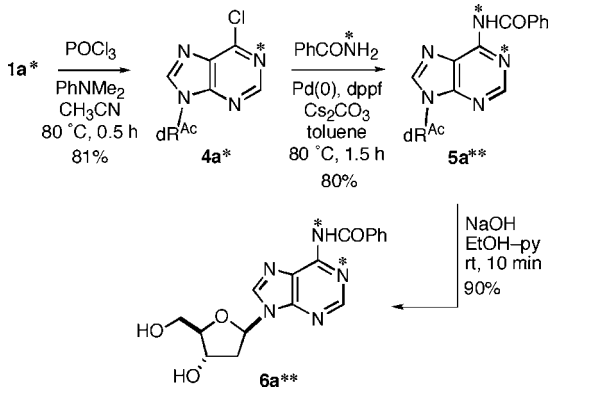
To bypass the two drawbacks mentioned in the two preceding paragraphs, **3a*** was separated from small amounts of **1a** and [$^{15}$N]nosylamide[8] and then heated in refluxing anhydrous $CH_3CN$ with 1.0 equiv of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) for 7 h to afford nosylamide and **1a*** (74% yield, not contaminated with **1a**, as shown by FABMS and NMR).[9]

Silyl-protected deoxyinosines **1b** and **1c**, prepared by standard procedures,[6b] were also converted to their Ns derivatives in excellent yields. However, the ring opening of **2b** and **2c** took place much more slowly than that of **2a** and afforded not so good results: after 40 h in 8:1 dioxane−$H_2O$, **2b** underwent 75% attack at C2 (to give **3b***, not shown in Scheme 2) and 25% attack at S; after 48 h in $CH_3CN$−dioxane−$H_2O$, **2c** underwent ca. 50% attack at C2 (to yield **3c***, not shown) and ca. 50% attack at S.

This fact might be explained by the lower solubility of **2b** and **2c** in the $CH_3CN-H_2O$ or dioxane−$H_2O$ polar media required to solubilize the $^{15}NH_4Cl$/KOH mixture (and to perform the addition under mild conditions);[10] conformational differences among **2a**, **2b**, and **2c** might also play a role. From a practical point of view, the Ac group (**2a**) is more appropriate than silyl protecting groups.

From **1a*** we prepared the corresponding doubly $^{15}$N-labeled 2′-deoxyadenosines (**5a**** and **6a****) according to Scheme 3. First of all, we attempted the reaction of **1a*** with

**Scheme 3**




(5) Milder nitrating mixtures checked by us were $Cu(NO_3)_2/Ac_2O$, $HNO_3/Ac_2O$, and $AgNO_3$/PhCOCl. For a review, see: Romea, P.; Aragonès, M.; Garcia, J.; Vilarrasa, J. *J. Org. Chem.* **1991**, *56*, 7038. That the sensitivity of deoxypurine nucleosides regarding the anomeric bond cleavage is larger than that of the other natural nucleosides is a well-known fact.

(6) For reviews of alternative uses of the Ns group, see: (a) Kan, T.; Fukuyama, T. *Chem. Commun.* **2004**, 353. (b) Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*; Wiley: New York, 1999.

(7) NsCl (*o*NsCl) is much cheaper than *p*NsCl. Moreover, the Ns group is cleaved more selectively when used as a protecting group, see: (a) Wuts, P. G. M.; Northuis, J. M. *Tetrahedron Lett.* **1998**, *39*, 3889. We disclose here another advantage of Ns vs *p*Ns (lower percentages of desulfonylation), as the *o*-nitro partially blocks the attack at the S of the sulfonyl group.

an excess of *N*-bromosuccinimide (NBS) and $P(NMe_2)_3$ (HMPT) in $CH_3CN$, followed by addition of an excess of LiBr and heating,[11] but this gave rise to complete depurination, as has been noted.[11,12] On the other hand, the method of Robins et al.[13] for the preparation of 6-chloroinosines and 6-chlorodeoxyinosines ($POCl_3$, *N*,*N*-dimethylbenzenamine), when applied to **1a***, afforded an 81% yield of the desired chloro derivative **4a***. This halopurine could be transformed under relatively mild conditions to adenosine derivative **5a**** (Scheme 3), in a remarkable 80% yield (an outstanding result taking into account that we were dealing with a chloro derivative instead of the usually more reactive bromo and iodo analogues), by treatment with 1.10 equiv of $PhCO^{15}NH_2$,[14] 5 mol % $Pd_2(dba)_3{\cdot}CHCl_3$, 15 mol % 1,1′-bis(diphenylphosphino)ferrocene (dppf),[15] and 140 mol % $Cs_2CO_3$ in toluene at 80 °C for 90 min.

Smooth saponification of the ester groups of **5a**** (by addition of dilute aqueous NaOH to a solution of the nucleoside in EtOH−pyridine) gave **6a**** in 90% yield.[16] The isotopic purity of **6a**** was confirmed to be >96% by HRMS (FAB) and by NMR (e.g., H2 and NH appear as doublets without any singlet signal in the middle; see Supporting Information). Compound **6a**** (as well as any related $N^6$-protected double-labeled adenosine that could be prepared in the same way) is suitable for its incorporation as a tag into oligodeoxyribonucleotide sequences (ODN, DNA fragments) after standard protection and activation protocols.

In summary, the $^{15}N$ labeling of N1 of 2′-deoxyinosines via a RORC mechanism is feasible in good yields, inexpensively, only by using the Ns group, among the many EWGs checked to date in our lab. Starting preferably from the diacetyl derivative and isolating the open intermediate, the desired $N^1$-labeled deoxyinosine has been obtained by using only 1.1 equiv of commercially available $^{15}NH_4Cl$. Hence, [N,1-$^{15}N_2$]-deoxyadenosines can be prepared, also in good yields, by using again only 1.1 equiv of $PhCO^{15}NH_2$ (obtained quantitatively from $^{15}NH_4Cl$), through a key Pd/dppf-catalyzed chloride-to-benzamide exchange.

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**Supporting Information Available:** Experimental procedures and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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(8) Crude yield of **3a*** was estimated to be 90−95% (by NMR). Separation by flash chromatography on silica gel (99:1 $CH_2Cl_2$−MeOH) afforded pure **3a*** in 80% yield. Relevant spectral data: $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 2.10 (s, 3 H), 2.11 (s, 3 H), 2.47 (ddd, $J$ = 14.0, 6.2, 3.0 Hz, 1 H), 2.54 (ddd, $J$ = 14.0, 7.7, 6.4 Hz, 1 H), 4.22−4.38 (m, 3 H), 5.31 (m, 1 H), 5.80 (br d, $J$ = 89.6 Hz, 2 H, C-*$NH_2$), 6.10 (dd, $J$ = 7.7, 6.2 Hz, 1 H), 7.47 (s, 1 H), 7.71−7.82 (m, 3 H), 8.38 (m, 1 H), 8.67 (br s, 1 H). $^{13}C$ NMR ($CDCl_3$, 100.6 MHz) $\delta$ 20.8, 20.9, 37.9, 63.7, 74.4, 82.2, 84.1, 117.8, 124.9, 130.3, 132.4, 132.7, 132.9, 134.3, 147.0, 148.2, 157.6 (d, $J$(C-*$NH_2$) = 18.3 Hz), 160.5, 170.3, 170.5.

(9) Other additives ($Et_3N$, $KOBu^t$) and reaction conditions gave rise to much lower cyclization yields. In refluxing $CH_3CN$, without DBU, the highest yield was 63%.

(10) Ring-opening reactions with $^{15}NH_4Cl/KOBu^t/NEt_3$ in anhydrous $CH_3CN$ turned out to be much slower, and byproducts coming from the attack at S were produced in higher percentages.

(11) (a) Véliz, E. A.; Beal, P. A. *J. Org. Chem.* **2001**, *66*, 8592. (b) Véliz, E. A.; Beal, P. A. *Tetrahedron Lett.* **2000**, *41*, 1695.

(12) With only 1.1 equiv of NBS and HMPT, in $CH_3CN$ at −20 °C, the phosphonium salt intermediate could be readily detected. The resulting solution was added via syringe to an excess of a saturated solution of $BnEt_3N^+Br^-$ in anhydrous $CH_3CN$ and slowly warmed to 70 °C; however, this afforded the corresponding 6-(dimethylamino)purine nucleoside as the major compound (not the desired bromo derivative).

(13) (a) Liu, J.; Janeba, Z.; Robins, M. J. *Org. Lett.* **2004**, *6*, 2917. (b) Janeba, Z.; Francom, P.; Robins, M. J. *J. Org. Chem.* **2003**, *68*, 989.

(14) Obtained by us in quantitative yield from only 1.1 equiv of $^{15}NH_4Cl$ and excesses of PhCOCl and $Et_3N$ (ref 3a).

(15) Purchased from Fluka. For classical works on the use of dppf, see: (a) Hayashi, T.; Konishi, M.; Kobori, Y.; Kumada, M.; Higuchi, T.; Hirotsu, K. *J. Am. Chem. Soc.* **1984**, *106*, 158 and references therein. For Pd-catalyzed couplings of amides, see: (b) Huang, X.; Anderson, K. W.; Zim, D.; Jiang, L.; Klapars, A.; Buchwald, S. L. *J. Am. Chem. Soc.* **2003**, *125*, 6653 and references therein. For Buchwald−Hartwig aminations with aryl and heteroaryl chlorides, see: (c) Urgaonkar, S.; Verkade, J. G. *J. Org. Chem.* **2004**, *69*, 9135 and references therein. (d) Shen, Q.; Shekhar, S.; Stambuli, J. P.; Hartwig, J. F. *Angew. Chem., Int. Ed.* **2005**, *44*, 1371.

(16) **Spectral Data of 6a**.** $^1H$ NMR (DMSO-$d_6$, 300 MHz) $\delta$ 2.36 (ddd, $J$ = 13.3, 6.2, 3.3 Hz, 1 H), 2.79 (ddd, $J$ = 13.3, 7.2, 6.3 Hz, 1 H), 3.49−3.67 (m, 2 H), 3.90 (m, 1 H), 4.45 (m, 1 H), 5.01 (t, $J$ = 5.7 Hz, 1 H), 5.36 (d, $J$ = 4.2 Hz, 1 H), 6.48 (dd, $J$ = 7.2, 6.2 Hz, 1 H), 7.51−7.64 (m, 3 H), 8.04 (d, $J$ = 7.5 Hz, 2 H), 8.68 (s, 1 H), 8.74 (d, $J$ = 15.6 Hz, 1 H), 11.17 (d, $J$ = 88.5, 1 H); $^{13}C$ NMR (DMSO-$d_6$, 100.6 MHz) $\delta$ 62.3, 71.4, 84.4, 88.7, 126.6 (d, $J$ = 1.0 Hz), 129.2, 133.1, 134.0 (d, $J$ = 7.8 Hz), 143.7, 151.0 (dd, $J$ = 18.3, 4.6 Hz), 152.2 (dd, $J$ = 4.0, 1.5 Hz), 152.6 (dd, $J$ = 4.0, 2.0 Hz), 167.3 (d, $J$ = 15.9 Hz); $^{15}NMR$ (DMSO-$d_6$, 30.4 MHz, concentrated $H^{15}NO_3$ as the external reference) $\delta$ −209.1 (d, $J$ = 88 Hz), −80.2 (d, $J$ = 14 Hz); HRMS ($FAB^+$) *m*/*z* found 358.1292, calcd for $C_{17}H_{18}{}^{14}N_3{}^{15}N_2O_4$ ($M + H^+$) 358.1299.