A novel nucleophilic approach to 1-alkyladenosines. A two-step synthesis of [1-^{15}N]adenosine from inosine†

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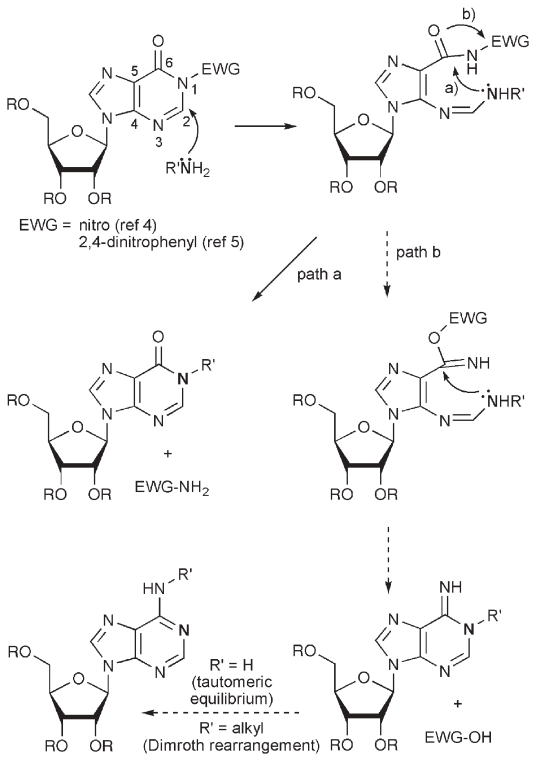
A novel ANRORC mechanism in the reaction of 1-(2,4-dinitrobenzenesulfonyl)inosines with amines has allowed the preparation of 1-alkyladenosines and [1-^{15}N]adenosines in a straightforward way from inosines.

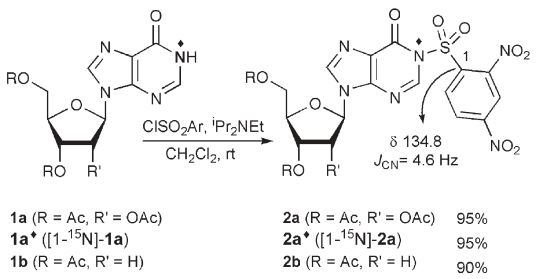
Alkylation of nucleic acids plays an important role in the etiology and treatment of cancer. N-Alkylated nucleosides are the primary origin of many carcinogenic processes caused by the interaction of alkylating agents with nucleic acids. These modified nucleosides avoid normal mitosis, interfere with transcription and in many cases induce apoptosis.[1] In the case of adenosine the main alkylated position is the more basic nitrogen (N^1).[2] Interestingly, N^1-alkylated adenosines are not only formed by the action of an external source. Thus, 1-methyladenosine is naturally formed by a methyltransferase enzyme and it is commonly found in the tRNA from all three biological domains (Eukaryota, Bacteria and Archaea).[3] Despite the inherent interest of these modified nucleosides, all the methodologies for their preparation are based on the nucleophilic attack of N^1 to electrophiles.[2] Herein we present the unprecedented reverse process where a nucleophilic amine is added to an electrophilic purine to obtain 1-alkyladenosines. Addition of amines to inosines has been documented and it is an easy way to obtain 1-alkylated inosines (Scheme 1, path *a*).[4,5]

The process involves addition of the amine to C^2 of an inosine that is activated with an electron-withdrawing group (EWG) at N^1. An open intermediate is formed that cyclises to the product. On the other hand, migration of the EWG to the O^6 (path *b*) might give rise to a different intermediate that would afford 1-alkyladenosines after cyclisation.

Looking for alternative EWG to the nitro group,[4] we explored the ability of 2,4-dinitrobenzenesulfonyl group in performing such processes. Thus, we prepared protected 1-(2,4-dinitrobenzenesulfonyl)inosines **2a** and **2b** by reaction of protected inosines **1a** and **1b** with 2,4-$(NO_2)_2C_6H_3SO_2Cl$ (DNsCl) and $^iPr_2NEt$ in CH_2Cl_2 at rt (Scheme 2).[6] We confirmed that sulfonylation occurred at N^1 instead of O^6 when we prepared sulfonylated [1-^{15}N]inosine **2a**$^\blacklozenge$ from [1-^{15}N]inosine **1a**$^\blacklozenge$.[4] The ^{13}C NMR spectrum of **2a**$^\blacklozenge$ compared to **2a** showed the splitting of some signals as a result of C–N couplings. Interestingly, we observed a coupling constant (J_{CN} = 4.6 Hz) at C^1, of the 2,4-dinitrophenyl moiety,[7] which clearly suggested that the sulfonyl group was attached to N^1.



Scheme 1 Nucleophilic amine addition to activated inosines.



Scheme 2 *N*-Sulfonylation of inosine.

Having in hand inosines **2**, our first experiments were directed to the addition of ^{15}N-labelled ammonia. Specific ^{15}N labelling 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.[8] Consistent with previous results with other EWG, we expected that addition of $^{15}NH_3$ to inosine **2a** would afford the corresponding [1-^{15}N]inosine

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according to path *a* in Scheme 1.[4,9] Therefore, we anticipated that addition of unlabelled ammonia should afford **1a**, either through path *a* or through desulfonylation by direct nucleophilic attack of NH_3 on the sulfur atom. However, when we added 1 equiv. of NH_3 we obtained significant amounts of a new compound that was chromatographically and spectroscopically identical to triacetyladenosine (**3a**).[10] Interestingly, addition of 1 equiv. of $^{15}NH_3$ (generated *in situ* from $^{15}NH_4Cl$ and base) to **2a** afforded labelled [1-^{15}N]adenosine **3a*** in 44% yield (Scheme 3).[11]‡ Analogous behaviour was observed for 2-deoxyinosine **2b**.

The appearance of the expected ^{1}H–^{15}N and ^{13}C–^{15}N couplings in the ^{1}H and ^{13}C NMR spectra of **3a*** confirmed that the label was on N^1. In addition, its proton-coupled ^{15}N NMR spectrum showed only a doublet ($J_{N,2}$ = 15 Hz) at δ −175.4 (10 M $H^{15}NO_3$ as the external reference).

Overall, the above protocol constitutes a straightforward approach (two steps from inosine **1a**) to the O-protected [1-^{15}N]adenosines by using 1 equiv. of the $^{15}NH_4Cl$ as a cheap label source.[12]

Furthermore, when we carried out the analogous reaction but with labelled [1-^{15}N]inosine **2a**$^{\blacklozenge}$ and non-labelled NH_4Cl the reaction proceeded similarly but the label now appeared exclusively at the exocyclic amino of adenosine (**3a**$^{\blacklozenge}$ in Scheme 4), as shown by the couplings observed in its NMR spectra. In this case the proton-coupled ^{15}N NMR spectrum showed a triplet (J_{NH} = 90 Hz) at δ −273.4.

Monitoring this reaction by TLC, we observed initially the formation of inosine **1a**$^{\blacklozenge}$ and a more polar intermediate. On heating, this intermediate was further transformed to the labelled [$^{15}NH_2$]adenosine **3a**$^{\blacklozenge}$.[13]

The above facts seemed to indicate that path *b* in Scheme 1 might be operating and, therefore, that the preparation of 1-alkyladenosines by this method might be possible. Actually, when we treated inosine **2a** with 1 equiv. of benzylamine (at low temperature to avoid desulfonylation) and we heated the resulting intermediate in a mixture of CH_3CN–H_2O,[14] we obtained a product in 81% yield that was identical to the benzyladenosine **4a** (Scheme 5).[15]§ Other amines such as ethylamine or butylamine showed a similar behaviour. Even the sterically more hindered isopropylamine gave the alkylated adenosine in good yield. Especially interesting is adenosine **4d** since it can not be obtained easily by a standard electrophilic alkylation.

The progress of these reactions showed also by TLC the formation of polar intermediates that were transformed into the products when heated.

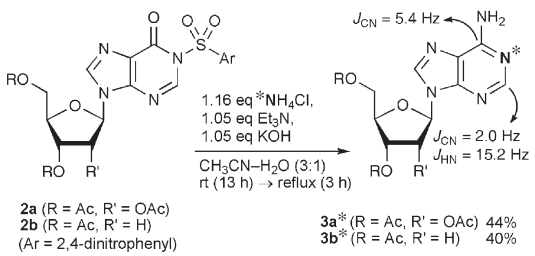
Surprisingly, under these conditions the product **4a** did not undergo a Dimroth rearrangement[16] to the corresponding 6-*N*-benzyladenosine **5a** (last step in path *b* of Scheme 1).[17] Only when compound **4a** was treated under harsh Dimroth rearrangement conditions (Me_2NH in refluxing CH_3CN) compound **5a** was obtained in 76% yield (Scheme 6).[18]

A remarkable fact in the formation of 1-alkyladenosines is that 2,4-dinitrophenol (**6**) is obtained in the same yields as the alkylated adenosines **4**. The formation of **6** might come from a S_NAr mechanism, through the attack of the amide-like oxygen atom on C^1 of the 2,4-dinitrophenyl moiety, as shown in Scheme 7.

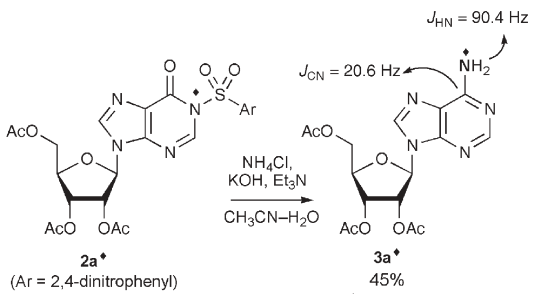
To evaluate this possibility we prepared [6-^{18}O]inosine **2a**$^{\diamond}$.[19] When we treated this labelled inosine (**2a**$^{\diamond}$) with benzylamine as above we obtained 89% of the fully labelled [1-^{18}O]-2,4-dinitrophenol (**6**$^{\diamond}$) besides adenosine **4a** in 86% yield. This unprecedented mechanism might be the responsible for the different behaviour of this EWG.

In our mechanistic proposal, we have excluded the possibility that the migration of the nitrophenyl moiety might occur prior the nucleophilic addition at C^2, because the product that would result (*i.e.* 6-*O*-arylinosine) usually adds nucleophiles at C^6.[20]

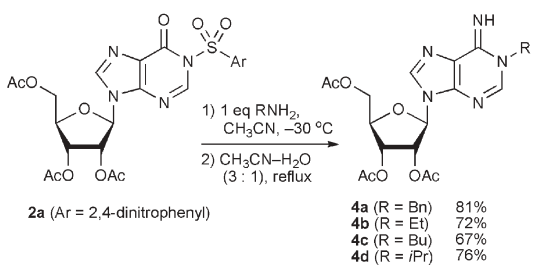
Nevertheless, the cyclisation through path *a* (Scheme 1) can be achieved using the conditions optimised for other EWG.[4] Thus, by addition of 2 equiv. of benzylamine to inosine **2a** in CH_3CN at low temperature and cyclisation by heating with 1 equiv. of CF_3CO_2H, we obtained a 62% yield of 1-benzylinosine **7a** (Scheme 8). Better yields were obtained with less sterically hindered amines such as methylamine and ethylamine.



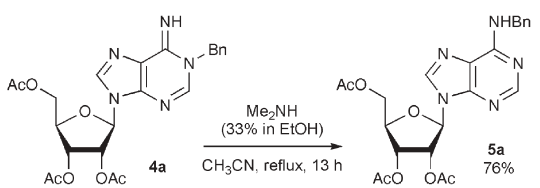
Scheme 3 [1-^{15}N]Adenosine formation from inosine.



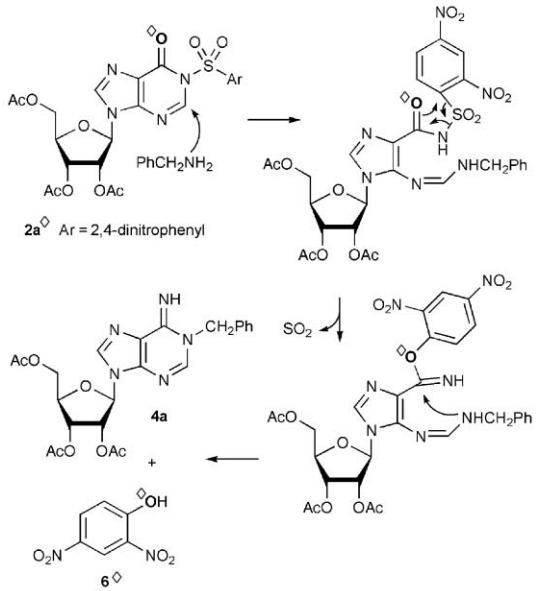
Scheme 4 [6-^{15}N]Adenosine formation from [1-^{15}N]inosine.



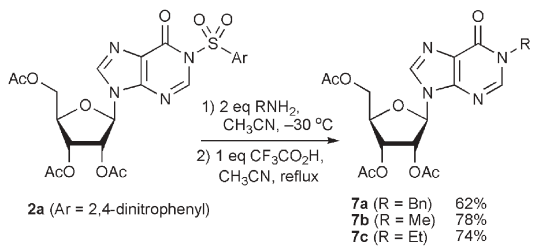
Scheme 5 Synthesis of 1-alkyladenosines.



Scheme 6 Dimroth rearrangement of **4a** to **5a**.



Scheme 7 Mechanism of formation of 1-alkyladenosine **4**.



Scheme 8 Synthesis of 1-alkylinosines.

In conclusion, the 2,4-dinitrobenzenesulfonyl group appears to be a very interesting activating group since it allows an easy transformation of inosines into 1-alkylinosines or into 1-alkyladenosines and [1-^{15}N]adenosines (by a unique ANRORC rearrangement). Currently, this method is being applied to the preparation of novel 1-alkyladenosines that might be pharmacologically active through their interaction with purine receptors.[21]

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Notes and references

‡ *General procedure* for the preparation of [1-^{15}N]adenosines: $^{15}NH_4Cl$ (1.16 mmol) and KOH (1.05 mmol) were placed in a round-bottomed flask sealed with a septum. Then, water (5 mL), CH_3CN (14 mL), Et_3N (1.05 mmol), and a solution of inosine **2a** or deoxyinosine **2b** (1.00 mmol) in CH_3CN (2 mL) were added sequentially *via* syringe. After vigorous stirring for 13 h, the reaction mixture was heated at reflux for 3 h. The resulting yellow solution was cooled to room temperature and the volatile materials were removed by rotatory evaporation. [1-^{15}N]Adenosines **3a*** were isolated by flash chromatography (CH_2Cl_2–MeOH from 98:2 to 95:5). Spectral data for **3a***: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.09 (s, 3H), 2.13 (s, 3H), 2.15 (s, 3H), 4.38 (dd, J = 5.4, 11.2 Hz, 1H), 4.43–4.47 (m, 2H), 5.67 (dd, J = 5.4, 4.6 Hz, 1H), 5.82 (br s, 2H), 5.93 (dd, J = 5.4, 5.3 Hz, 1H), 6.18 (d, J = 5.3 Hz, 1H), 7.97 (s, 1H), 8.37 (d, J = 15.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4, 20.5, 20.7, 63.1, 70.6, 73.2, 80.3, 86.2, 120.1 (d, J = 2.5 Hz), 138.9, 149.8 (d, J = 3.0 Hz), 152.9 (d, J = 2.0 Hz), 155.3 (d, J = 5.4 Hz), 169.4, 169.6, 170.3. ^{15}N NMR ($CDCl_3$, 30 MHz): δ −175.4 (10 M $H^{15}NO_3$ as the external reference). HRMS (FAB): calc. for $C_{16}H_{20}N_4{}^{15}NO_7$ $(M + H)^+$ 395.1333, found 395.1333.

§ *General procedure* for the preparation of 1-alkyladenosines: In a two-necked flask, inosine **2a** (1.00 mmol) was solved in CH_3CN (12 mL). Then, a solution of the alkylamine (1.00 mmol) in CH_3CN (12 mL) was added *via* cannula at −30 °C and stirring was continued until the starting nucleoside was consumed, as determined by TLC analysis (typically 30 min). Afterwards, water (8 mL) was added and the reaction mixture was heated at reflux. When the consumption of the intermediate (around 40 min) was completed, the resulting yellow solution was allowed to cool to room temperature and concentrated *in vacuo*. The 1-alkyladenosine **4** was isolated by flash chromatography (CH_2Cl_2–MeOH from 99:1 to 95:5). Spectral data for **4a**: ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.09 (s, 3H), 2.12 (s, 6H), 4.30–4.43 (m, 3H), 5.26 (s, 2H), 5.61 (dd, J = 5.5, 4.8 Hz, 1H), 5.86 (dd, J = 5.5, 5.0 Hz, 1H), 6.00 (d, J = 5.0 Hz, 1H), 7.30–7.36 (m, 5H), 7.73 (s, 1H), 7.74 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 20.4, 20.5, 20.7, 49.9, 63.0, 70.4, 73.2, 80.1, 86.5, 124.4, 127.8, 128.0, 128.9, 136.0, 136.9, 141.3, 147.7, 154.5, 169.3, 169.5, 170.3. HRMS (FAB): calc. for $C_{23}H_{26}N_5O_7$ $(M + H)^+$ 484.1832, found 484.1833.

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