COMMUNICATION

Diversely C8-functionalized adenine nucleosides *via* their underexplored carboxaldehydes†

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Hari K. Akula,[a] Suyeal Bae,[a] Padmanava Pradhan,[a] Lijia Yang,[a] Barbara Zajc*[ab] and Mahesh K. Lakshman*[ab]

The potentially versatile *N*-unprotected 8-formyl derivatives of adenosine and 2'-deoxyadenosine are highly underexploited for C8 modifications of these nucleosides. Only *in situ* formation of 8-formyladenosine is known and a single application of an *N*-benzoyl derivative has been reported. On the other hand, 8-formyl-2'-deoxyadenosine and its applications remain unknown. Herein, we report straightforward, scalable syntheses of both N-unprotected 8-formyladenine nucleoside derivatives, and demonstrate broad diversification at the C8 position by hydroxymethylation, azidation, CuAAC ligation, reductive amination, as well as olefination and fluoroolefination with modified Julia and a Horner-Wadsworth-Emmons reagents.

Nucleosides constitute an exceptionally important class of biomolecules, present in all living organisms. Due to their ubiquity, modified nucleosides have found wide-spread applications as biological probes, in biochemistry, and in medicine. For instance, base-modified fluorescent nucleosides can be used to probe microenvironment in DNA and RNA, base-base interactions, and structure-function relationships.[1–9] Modified nucleosides are also at the forefront in the control and treatment of existing as well as emerging viral diseases and cancer.[9–16]

Generally, the most relied upon approach for the introduction of a "carbon substituent" at the C8 position of adenine nucleosides commences from the 8-bromo[17,18] or iodo nucleoside analogues.[19] Known metal-catalyzed reactions with these halo derivatives include alkynylation for generating Csp bonds,[20–24] Heck-like[25,26] and Suzuki-Miyaura-type reactions to introduce Csp^2 linkages.[18a,27–29] Introduction of alkyl, allyl, and vinyl groups has been accomplished by cross coupling with Grignard,[30] organotin,[19,31] and organoaluminum reagents.[32] Access to C8 alkenyl and alkyl adenosine analogues has been attained by either partial or complete reduction of alkynyl derivatives, respectively. Reaction of C8 lithio adenosine derivatives with an appropriate electrophile offers access to C8 alkyl derivatives.[33,34] Alternatively, deprotonation of a 8-(ethoxycarbonylmethyl)adenosine derivative, obtained in three steps from 2',3'-*O*-isopropylidene-8-bromoadenosine, followed by alkylation and decarboxylation or just decarboxylation was an alternate route to 8-alkyl adenosine derivatives.[35,36]

In the light of the foregoing discussion and because of the need for new approaches to enable diverse nucleoside modifications, we reasoned that novel segue to C8 functionalization of adenine nucleosides could be attained *via* their 8-formyl derivatives. In the prior literature, lithiation at the C8 position of silyl-protected adenosine by LDA followed by reaction with HCO_2Me was reported to lead to the 8-aldehyde.[34] However, this was not isolated but was directly reduced to the alcohol with $NaBH_4$.[34] Lithiation of the silyl-protected antibiotic cordycepin (3'-deoxyadenosine) with LDA and reaction with HCO_2Me gave three products. Two returned to starting material upon treatment with NH_3/MeOH and the third was the 8-formyl derivative (36% yield).[37]

It is quite possible that on account of the undesired *N*-formylation of the nucleobase, a singular report described the synthesis of an *N*-benzoyl 8-formyladenosine derivative and its use in one reaction with an iminophosphorane.[38] The *N*-benzoyl group was ultimately removed with NaOMe in MeOH.[38] However, for many applications, *N*-protection and deprotection represent unnecessary additional steps, not considering undesirable reactions at other functionalities that may be present.

On the basis of these considerations, we set out to reassess C8 lithiation/formylation of precursor **1** and then **2**, the latter being unknown. First, lithiation of 2',3',5'-tri-*O*-TBS-protected adenosine (**1**, Scheme 1) with 5 eq. of LDA in THF at –78 °C and reaction with 6 eq. of HCO_2Me, gave two products. The major was the C8,N^6-diformyl derivative **3**, whereas the 8-aldehyde **4**

[a] Department of Chemistry and Biochemistry, The City College of New York, 160 Convent Avenue, New York, NY 10031, USA. E-mail: bzajc@ccny.cuny.edu, mlakshman@ccny.cuny.edu
[b] The PhD Program in Chemistry, The Graduate Center of the City University of New York, New York, NY 10016, USA

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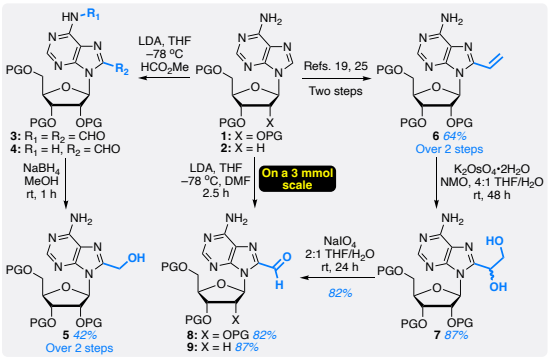
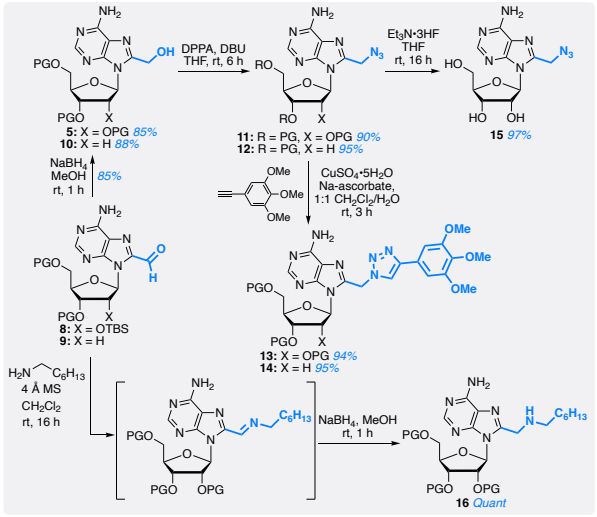
was minor. This relative product distribution remained unchanged even with 7 eq. each of LDA and HCO_2Me. With 7 eq. of LDA and 10 eq. of HCO_2Me, only diformyl derivative **3** was observed. In all cases, the crude products were reduced with $NaBH_4$ in MeOH to furnish adenosine carbinol **5**. The best yield of compound **5** (42% over two steps) was obtained from a reaction with 7 eq. of LDA and 10 eq. of HCO_2Me. Although carbinol **5** can potentially be oxidized to aldehyde **8**, because of the modest yield, other methods were investigated. One of these relied on the iodination/vinylation of tri-*O*-TBS-protected adenosine **1**.[19,25] Dihydroxylation of 8-vinyladenosine derivative **6** gave a diastereomeric pair of diols **7**, which upon reaction with $NaIO_4$ yielded 8-formyladenosine **8** in a 46% yield over four steps. Because this was still below acceptable, lithiation of nucleoside **1** with 5 eq. of LDA in THF and reaction with 25 eq. of DMF was tested. Gratifyingly, the 8-formyl derivative was *directly* obtained in a high 82% yield after purification, and without any complicating side reactions. The method was equally applicable to the more labile 3',5'-di-*O*-TBS-protected 2'-deoxyadenosine **2**, yielding the unknown 8-formyl deoxynucleoside **9**, in an 87% isolated yield. The reactions are relatively fast and readily scalable to 1–3 mmol of substrate.



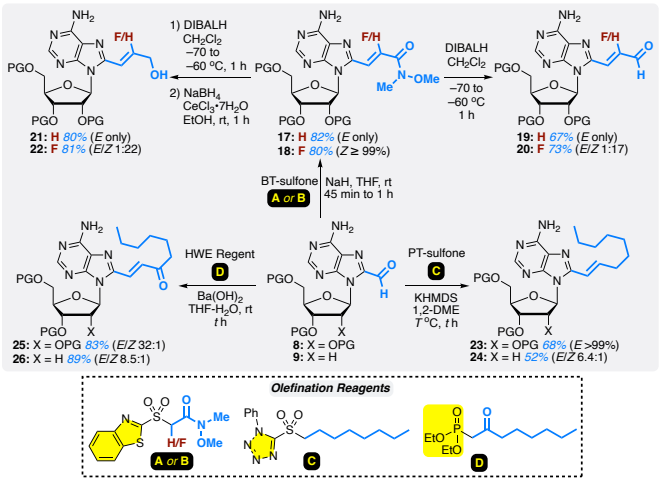
Scheme 1 Synthesis of silyl-protected 8-formyl derivatives of adenosine and 2'-deoxyadenosine (PG = *t*-$BuMe_2Si$)

With the 8-formyladenine nucleosides in hand, the next focus was assessing their participation in diverse applications (Scheme 2). $NaBH_4$ reduction of aldehyde **8** gave the known alcohol **5**,[34] whereas aldehyde **9** gave the unknown alcohol **10**. A one-step azidation of each[39] led to azides **11** and **12**. Because azides are excellent partners in CuAAC reactions, these azides were reacted with 3,4,5-trimethoxyethynylbenzene under modified conditions, to prevent a previously noted reduction of a nucleoside azide to the amine under CuAAC conditions.[40] Compounds **13** and **14**, with an attached biologically relevant combretastatin A4 unit, were obtained in excellent yields. Whereas azide **11** underwent desilylation to the 8-azidomethyl ribonucleoside **15** with *n*-$Bu_4N^+F^-$, within 1 h, extensive product degradation occurred upon chromatography, including with deactivated silica (best yield 37%). This is yet one more instance of the sensitivity of nucleoside-derived products, in contrast to simpler systems. Use of $Et_3N•3HF$, on the other hand, eliminated this problem and simply washing the product with CH_2Cl_2 yielded azidomethyl derivative **15** in a high yield and purity. Finally, reductive amination of adenosine-8-carboxaldehyde **8** with *n*-heptyl amine gave an excellent yield of alkyl amino derivative **16**, without complications at the free adenosine amino group.



Scheme 2 Transformations of silyl-protected 8-formyl derivatives of adenosine and 2'-deoxyadenosine (PG = *t*-$BuMe_2Si$)

We next considered another important transformation, olefination chemistry, using modified Julia (benzothiazolyl: BT, 1-phenyltetrazolyl: PT) and Horner-Wadsworth-Emmons (HWE) reagents, and further conversions of some of these products. First, aldehyde **8** was reacted with BT-sulfone **A** and its fluoro analogue **B**.[41,42] These reactions, leading to products **17** and **18**, proved to be quite straightforward with NaH in THF at room temperature, and proceeded in good to high yields. Notably, exclusive *E* selectivity was observed with reagent **A** and exclusive *Z* selectivity was observed with reagent **B**. Weinreb amides **17** and **18** could be partially reduced to novel nucleoside enals **19** and **20,** as well as allylic alcohols **21** and **22**, all of which are additionally functionalizable. In these experiments, some isomerization was observed with the fluoro olefins, either in the reactions, or workup, or chromatography. This isomerization was not observable with the protio analogues. Olefin isomerization of C8 styryl adenosine derivatives has previously been reported.[25]



Scheme 3 Olefination and fluoroolefination reactions of silyl-protected 8-formyl derivatives of adenosine and 2'-deoxyadenosine (PG = *t*-$BuMe_2Si$)

Reactions with PT-sulfone **C** needed optimizations (see the ESI). On a small scale (0.078 mmol of aldehyde **8**), reactions with

PT-sulfone **C** (2 eq.) under Barbier conditions at –78 °C, using Li or Na or KHMDS (3 eq.) in 2:1 THF-PhMe, gave low conversions (21%, 33%, and 42%, respectively). Among the three bases, KHMDS was superior but increasing the amount of KHMDS to 7 eq. only led to a marginal increase in conversion (45%). Use of KHMDS (5 eq.) in 1,2-DME[43] at –78 °C gave a dramatic improvement (83% conversion). High conversions on both the 0.078 and 0.31 mmol scales were observed when the reaction was initiated at –78 °C, warmed to –30 °C, and then to room temperature. At the higher scale, reaction of ribose derivative **8** was complete, whereas that of 2'-deoxyribose **9** was 93% complete. Yields of the product olefins **23** and **24** were 68% and 52%, respectively, with high *E* selectivity observed in both cases. With aldehyde **8** only a trace amount of the *Z* olefin was observed and with aldehyde **9**, the *E*/*Z* ratio was 6.4:1.

Reactions with HWE reagent **D** also necessitated optimizations (see the ESI). With a 1:1 ratio (0.078 mmol each) of 8-formyladenosine derivative **8** and phosphonate **D**, and with $Ba(OH)_2$ in 40:1 THF-H_2O,[44,45] 85–86% yields of enone **25** were obtained within 1 h at room temperature. Scaleup to 0.31 mmol caused the mixture to become a gel, requiring dilution of the reaction mixture, and leading to ca. 90% conversion over 1 h. Workup and re-exposure to 0.2 eq. each of reagent **D** and $Ba(OH)_2$ led to complete consumption of aldehyde **8** and formation of enone **25** in an 83% yield (*E*/*Z* = 32:1). With 8-formyl-2'-deoxyadenosine derivative **9**, similar effects were observed. On the 0.078 mmol scale, with 1.2 eq. each of phosphonate **D** and $Ba(OH)_2$, complete reaction was observed within 4 h (82% yield of enone **26**). Scaleup to 0.31 mmol, caused the reaction mixture to become a gel, requiring dilution and leading to ca. 90% conversion. As with the reaction of the ribose derivative **8**, workup of the reaction mixture and re-exposure to 0.2 eq. each of phosphonate **D** and $Ba(OH)_2$ led to complete consumption of aldehyde **9** and the formation of enone **26** in an 89% yield (*E*/*Z* = 8.5:1).

Several products in Scheme 3 are Michael acceptors and, as mentioned earlier, this motif could pose problems in the event an *N*-acyl protecting group requires nucleophilic cleavage. Thus, the precursors herein eliminate such problems. Weinreb amides generally provide segue to structural diversification, as exemplified by the ~~two~~ examples in Scheme 3. Notably, these amides can yield other nucleoside-based Michael acceptor derivatives that could be potentially useful in therapeutic design. A large number of protein kinases have cysteine residues in and proximal to a conserved ATP binding site. Thus, nucleosides bearing Michael acceptor motifs can function as soft electrophiles for reactions with the thiol moiety of cysteine residues, while targeting the adenine-binding site. Examples of Michael acceptor-containing anticancer compounds are the FDA-approved ibrutinib, neratinib, and lumakras, as well as a fluorovinyl amide-containing KRAS inhibitor, MRTX849, that is in clinical trials.

Quantum mechanical calculations have been utilized to understand soft-soft interactions, such as those between sulfhydryl groups and conjugated olefins.[46] Therefore, we decided to evaluate the HOMO and LUMO energies of enals **19** and **20** (as the unprotected versions) by DFT at the B3LYP/611-G++(d,p) level (see Figure 1). In these assessments, the LUMO energy of the fluorinated enal was substantially lower in comparison to the protio analogue. With these, we calculated the softness parameter σ and electrophilicity index ω of the compounds (see the ESI for additional details). The σ values for enals **19** and **20** were 0.547 eV^{-1} and 0.563 eV^{-1}, respectively. The electrophilicity indices ω for these compounds were 6.25 eV for enal **19** and 6.59 eV for fluoro enal **20**.

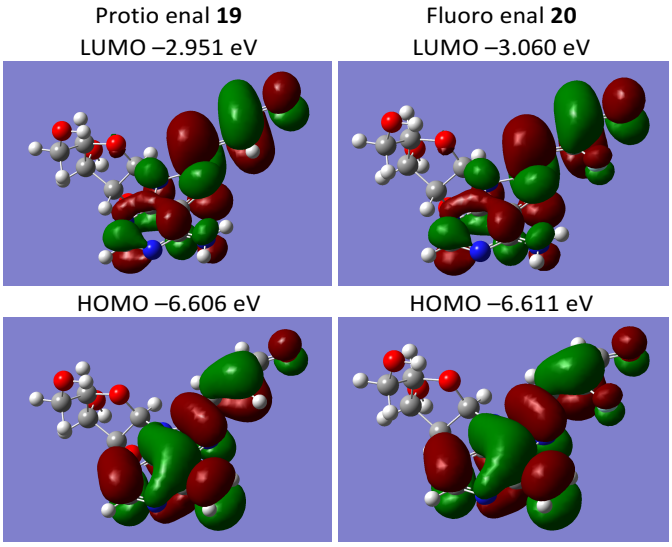


Fig. 1 Computed HOMO and LUMO orbitals of enals **19** and **20** (with free sugar hydroxyl groups).

Comparable analysis of the Weinreb enamides (with unprotected hydroxyl groups) showed a σ value of 0.520 eV^{-1} for protio analogue **17** with an ω index of 5.04 eV. The σ value for fluoro olefin **18** was 0.519 eV^{-1} and the ω index was 4.95 eV (see the ESI for additional details) These results were surprising, as we anticipated the fluorine atom to substantially influence the olefin softness and electrophilicity, as with the enals.

In summary, we have developed a one-step synthesis of 8-formyladenosine and 2'-deoxyadenosine, as their silyl-protected derivatives and devoid of an *N*-protecting group. Whereas synthesis of one *N*-acyl 8-formyladenosine derivative and a single reaction involving it has been reported,[38] the 2'-deoxyribose analogue is unknown. Therefore, these important biomolecular building blocks for chemical biology and medicinal chemistry applications have hitherto remained largely unexploited. The facile, scalable synthesis of both 8-formyladenine nucleosides and the demonstrated elaborations with each, create a platform for diverse further utilities. The azidomethyl derivatives, obtained *via* the nucleoside carbinols, can be readily utilized in CuAAC reactions and direct reductive amination of the aldehyde is straightforward. These nucleoside aldehydes are also substrates for olefination reactions, demonstrated *via* the use of three types of precursors; benzothiazole- and 1-phenyltetrazole-based modified Julia reagents, and a HWE reagent. In this context, we have seamlessly combined the ability to introduce a fluorine atom into potentially biologically valuable alkenes, setting up a scenario to be able to manipulate molecular energetics. These syntheses show no necessity for amino group protection and deprotection steps, where the latter could pose problems with

nucleophile-sensitive functionalities in the products. We anticipate that this disclosure will enable substantial novel diversification of these nucleoside scaffolds. In our future work we plan disclosure of diversified products and assessments of relevant biological results.

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Conflicts of interest

There are no conflicts to declare.

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