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COMMUNICATION

Synthesis and biological evaluation of unprecedented ring-expanded nucleosides (RENs) containing the imidazo[4,5-*d*][1,2,6]oxadiazepine ring system†

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A small collection of ring-expanded nucleosides (RENs), containing the unprecedented bis-alkylated imidazo[4,5-*d*][1,2,6]oxadiazepine heterocyclic ring system, has been synthesized through a new general approach. Results of preliminary cytotoxicity tests on breast (MCF-7) and lung (A549) cancer cell lines are also reported.

Nucleosides containing a 5 : 7-fused ring system as a nucleobase, also known as ring-expanded nucleosides (RENs), are very interesting molecules from several points of view. They are mimics of purine nucleosides and most of them possess significant biological activities due to their interference with the enzymes involved in the purine metabolism.[1] Coformycin and pentostatin (2′-deoxycoformycin) are two naturally occurring RENs containing an imidazo[4,5-*d*][1,3]diazepine ring system. They show a potent antitumor activity due to the strong inhibition of adenosine deaminase (ADA), acting as transition-state analogues.[2,3] Pentostatin is a drug approved in 1991 by FDA for the treatment of hairy cell leukemia. However, it is characterized by a severe toxicity that limits its use in therapy.[1] A number of coformycin analogues have been synthesized, both to investigate the structure–activity relationship and to produce less toxic derivatives.[4–9] Furthermore, imidazo[4,5-*c*]azepine nucleosides, ring-expanded derivatives of xanthosine, guanosine and inosine, have been investigated and their cytotoxic activity has been reported.[10] RENs have shown antiviral properties as well. Coformycin and some of its derivatives are able to enhance the activity of therapeutic antiviral drugs such as 2,3-dideoxyadenosine (ddA) and 2′-fluoro-2′,3′-dideoxyadenosine (FddA). Other RENs, for example those containing the 6-alkylamino-substituted imidazo[4,5-*e*][1,3]diazepine or the imidazo[4,5-*e*][1,2,4]triazepine moiety, show direct antiviral activity against human hepatitis B virus and West Nile Virus.[8]

Due to their noncanonical steric hindrance, RENs have been inserted in oligonucleotides to study conformational and stability changes in duplex, triplex and other DNA secondary structures, as well as the H-bonding profile, base stacking, *endo*/*exo* sugar puckering and the α/β anomeric ribose configuration.[11] From a synthetic point of view, the construction of a nucleoside containing a 5 : 7-fused ring base system can be performed using a sugar unit bearing a functionalized imidazole (or other 5-membered cycles), on which the seven-membered ring closure can be accomplished by inserting an appropriate molecular moiety.[9,10,12]

Alternatively, a pre-synthesized 5 : 7-fused heterocyclic system can be joined to a sugar moiety by reaction with an activated glycosyl donor.[7,13,14] The latter approach is longer but it does not suffer from problems due to the weakness of the *N*-glycosidic bond and it also offers the chance to synthesize both α/β nucleoside isomers.

In this communication we report the synthesis of new ring-expanded nucleosides **7a–c** (Scheme 1) containing the unprecedented imidazo[4,5-*d*][1,2,6]oxadiazepine heterocyclic system as the base moiety through a new approach featuring the opening/reclosure of the pyrimidine ring system of 2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl) nebularine N1-oxide **1**. Our synthetic strategy allowed us to prepare a small set of 5,8-dialkyl-(aryl)-substituted imidazo[4,5-*d*][1,2,6]oxadiazepine nucleosides (**7a–c**), in high yields, exploiting the previously reported reactivity of **1** towards Grignard reagents.[15a]

The reactivity of α-carbons towards Grignard reagents in aromatic nitrones has previously been observed for pyridine *N*-oxide[16–18] which results in cleavage of the N–C α bond with the concomitant formation of oxime and *trans*-alkene functions. In our previous studies[15a] we observed that reaction of nitrone **1** with a Grignard reagent leads to addition at C6 furnishing the adducts **2**, as a mixture of C6 diastereomers, which slowly decomposed on standing, partially dehydrating to C6-alkyl(aryl)nebularines **3**.

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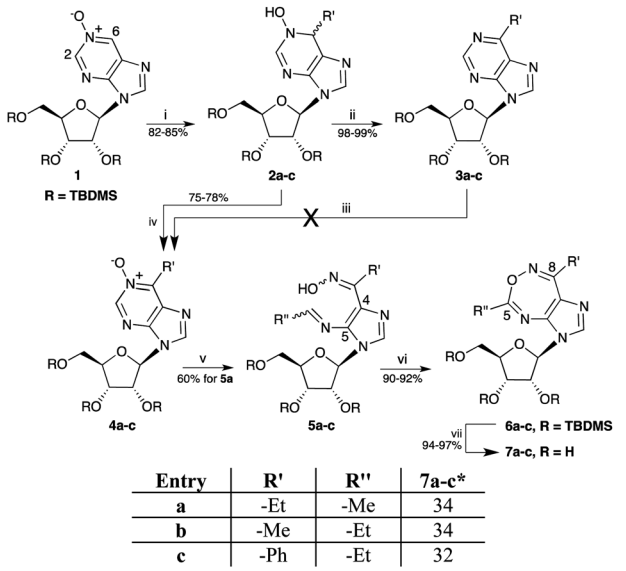
† Electronic supplementary information (ESI) available: Experimental procedures, compound characterization data, copies of ^{1}H- and ^{13}C-NMR spectra of compounds **4**, **6** and **7**, copies of UV spectra of compounds **4** and **6**, biological assays of compounds **7**. See DOI: 10.1039/c2cc33511e



Entry	R'	R''	7a-c*
a	-Et	-Me	34
b	-Me	-Et	34
c	-Ph	-Et	32

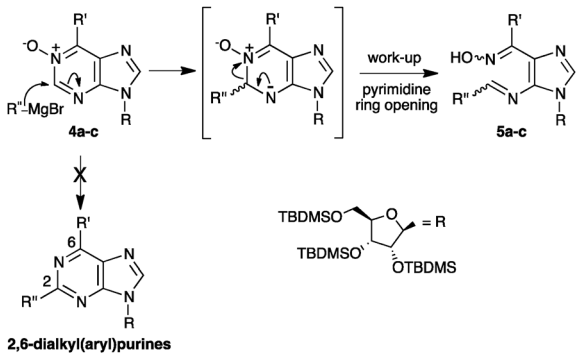
Scheme 1 *Reagents and conditions*: (i) R′MgBr (2 equiv.), THF, 2 h, rt; (ii) Ac_2O–pyridine (6 : 4), 1 h, 50 °C; (iii) $MeReO_3$–H_2O_2 (30% aq.), MeOH, 15 h, rt; (iv) pyridine, 16 h, 70 °C; (v) R″MgBr (4 equiv.), THF, 2 h, rt; (vi) ^{t}BuOOH (10 equiv.), CCl_4, reflux, 1 h; (vii) NH_4F (10 equiv.), MeOH, 5 h, reflux. *Reaction yields (%) from **1**.

This process was pushed towards aromatization by treatment of the crude adduct **2** with Ac_2O in pyridine at 50 °C, to give **3** in nearly quantitative yields.[15]

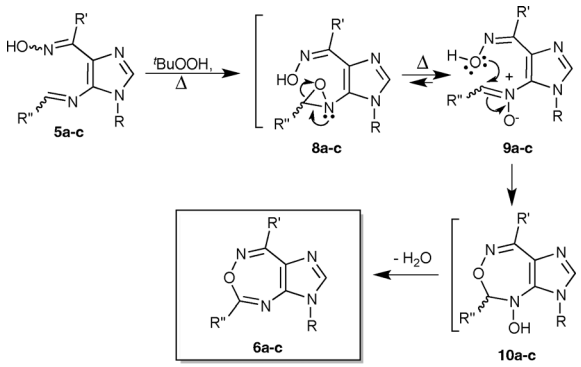
We reasoned that the introduction of a N1-nitrone function on **3** could induce an electrophilic behaviour of C2 as well, thus allowing us to obtain, at least in principle, 2,6-dialkylpurine. To probe this hypothesis the representative compound **3a** was treated with catalytic amounts of methyltrioxorhenium ($MeReO_3$) in the presence of H_2O_2, the same oxidizing mixture used to obtain nitrone **1** from silylated nebularine. Under these conditions, **3a** produced only traces of N1-oxide **4a**. Alternatively, the oxidation of the N1-hydroxy derivative **2a** with air in pyridine at 70 °C afforded the C6-ethylnebularine N1-oxide **4a** in 78% yield. The similar procedure using DMF as a solvent was reported for 2,6-alkyl(aryl)-substituted *N*-hydroxypyridine derivatives.[18]

The successive reaction of **4a** with Grignard reagents confirmed the electrophilic reactivity of C2 but, in this case, the addition was followed by the breaking of the N1–C2 bond leading to compound **5a** (60% yield) as a 1:1 mixture of (*E*/*Z*) isomers at the imine double bond (Scheme 2). In particular, 2D NMR experiments and HRMS data confirmed the structure of **5a**. The HMBC experiment supported the presence of the aldimine moiety showing clear correlations between the aldimine proton and both the aldimine carbon and the Grignard residue R″. Furthermore, the absence of correlation between aldimine proton and C6 of the purine ring strongly suggested the opening of the cycle at the N1–C2 bond. Next, we tested the reactivity of the open system of **5a** for the possible ring reclosure. In particular, treatment of **5a** with ^{t}BuOOH (10 equiv.) in refluxing CCl_4 gave nucleoside **6a**, containing the imidazo[4,5-*d*][1,2,6]-oxadiazepine framework (Scheme 3) in a 92% yield.

The accomplishment of the seven-membered ring could be explained by invoking the formation of the oxaziridine **8a** (Scheme 3) through oxidation of the aldimine functionality.[19]



Scheme 2 Proposed mechanism for the formation of compounds **5a–c**.



Scheme 3 Proposed mechanism for the formation of compounds **6a–c**.

It is reported that nucleophiles usually open the oxaziridine ring by attack at the nitrogen or oxygen[20] atom rather than on the carbon atom. However, it is known that oxaziridines containing aromatic substituents could easily rearrange to nitrones upon heating,[21] making the carbon the preferred attack site. Accordingly, nitrone **9a**, derived from **8a**, would undergo a fast ring reclosure by nucleophilic attack of the oxime hydroxyl function‡,[22] on the nitrone carbon,[23] to give intermediate **10a** (not isolable) which would lead to the oxadiazepine cycle **6a** by water loss.

Previous mechanistic studies on the reactivity of the 2-halo-quinazoline 3-oxide system with alkali[24] showed that the opening of the pyrimidine ring by nucleophilic attack of the hydroxyl ion at C2 afforded an *anti*-oxime. In our case, 2D-NMR studies of compound **5a** performed in pyridine-d_5 did not give conclusive information on the oxime configuration, although the (*Z*) configuration is needed for the ring closure (**9a** → **10a**).

The same reaction sequence leading to **6a** furnished compounds **6b–c** in comparable yields to **6a** (Scheme 1). Compounds **5a–c** are rather unstable and decompose on standing within a few hours. Therefore, reactions of **5b** and **5c** were performed in the crude reaction mixture without observing lowering of the yields.

Finally, nucleosides **6a–c** were deprotected at ribose using NH_4F in MeOH[25] to give, after HPLC purification, compounds **7a–c** (94–97% yield, Scheme 1).

The structure of nucleosides **6a–c** and **7a–c** was confirmed by 2D-NMR experiments and HRMS data. Moreover, the disappearance of the characteristic purine band around 260 nm in the UV spectra of **6a–b** (see ESI†) provided further evidence for the 7-membered ring formation, excluding the presence of the isomeric 2,6-disubstituted purine N1-oxide system.

Considering that RENs significantly inhibit the growth of several cancer cell lines,[1] we tested the cytotoxicity of the novel RENs by treating lung A549 and breast MCF-7 cancer cell lines with different concentrations (0.1, 1.0, 10 and 100 μM) of **7a–c**. At different time points for each concentration the cell viability was determined by measuring the mitochondrial activity.[26] Cisplatin was used as the control since its activity in these cell lines has been extensively studied.[27,28] We found that at day 7 all tested compounds significantly reduced the cell viability of breast cancer derived MCF-7 cells at the highest tested concentration (also at 10 μM in the case of **7a**). An explanation for such a delayed activity could be the slow formation of an active metabolite during the assay. At the same time point the inhibition of A549 cells growth, albeit observed, was only marginal (Fig. S1, MCF-7 cell line and Fig. S2, A549 cell line in ESI†).

In conclusion, we have proposed new reactions of the purine nucleobase enlarging the synthetic toolbox to obtain novel nucleoside analogues. The reactivity of the herein proposed C6-alkyl(aryl)purine-N1-oxides towards Grignard reagents afforded the new 4,5-disubstituted imidazo-nucleosides **5** from which the unprecedented imidazo[4,5-*d*][1,2,6]oxadiazepine nucleosides were built in good yields.

Moreover, it is to be noted that this heterocyclic system has not been so far reported and the proposed synthetic strategy allows the introduction of C-substituents on C5 and C8 of the oxadiazepine cycle. Finally, we have reported that new RENs are able to inhibit the cell growth of breast cancer derived MCF-7 cells. The observed cytotoxic effects of **7a–c** were dose-dependent and comparable to those of cisplatin.

Further studies to enlarge the collection of imidazo[4,5-*d*]-[1,2,6]oxadiazepine nucleosides, to investigate the reactivity of nebularine N1-oxide toward other C-nucleophiles, including organo-lithium reagents, as well as of the open intermediates **5a–c** will be carried out.

Notes and references

‡ The nitrogen of the oxime requires harsh conditions to react, as outlined in ref. 22.

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