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Synthesis of 1-pyrroline 1-oxides analogous to pseudouridine

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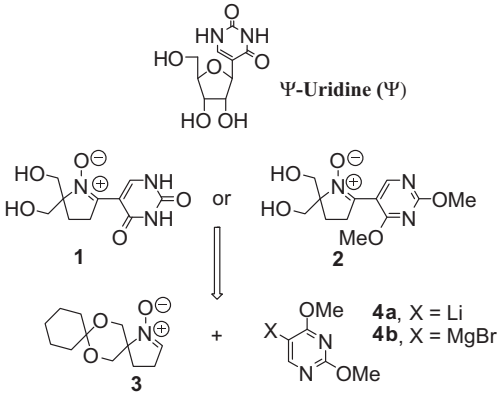
ABSTRACT

Pseudouridine (ψ-uridine, Ψ) aza′-analogues with a 5,5-bis(hydroxymethyl)-1-pyrrolin-2-yl 1-oxide as the glycone mimic were obtained by the addition of (2,4-dimethoxypyrimidin-5-yl)magnesium bromide to 1-aza-7,14-dioxadispiro[4.2.5.2]pentadec-1-ene 1-oxide (**3**), followed by oxidation and removal of the protecting groups. The analogous synthesis from (2,4-dimethoxypyrimidin-5-yl)lithium and **3** was less efficient; in the first step of the reaction sequence, competing dimerisation of **3** predominated over addition of the organolithium agent to **3**.

Pseudouridine (ψ-uridine, Ψ, Scheme 1) is a natural *C*-nucleoside occurring in various types of RNA. It has been generally accepted that Ψ plays a significant role in fine-tuning of RNA functions and preservation of its structure during the translation process.[1] Moreover, the anti-mutagenic activity of Ψ against ionizing radiation or chemical mutagens, such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine or *N*-methyl-*N*-nitrosourea, has also been reported.[2] It has been suggested that its activity towards the chemical mutagens is due to the entrapment of free radicals derived from these mutagens.[3a] The biological significance of Ψ has stimulated many studies on the synthesis of Ψ analogues as potential antiviral and/or anticancer agents. Among the analogues, derivatives with an aza-heterocyclic mimic of the natural glycone have also been reported.[3] The following aza-heterocycles have been reported in the role of the glycone mimic: pyrrolidine,[3a–k] oxazole,[3l] isoxazole,[3m] isoxazoline,[3m] isoxazolidine,[3m,n] imidazolidine,[3o] thiazolidine[3p] or 1,2,4-oxadiazole.[3q] Among these aza-heterocyclic Ψ analogues, pyrrolidin-2-yl derivatives were examined for their anti-HIV activity[3a,b] or base-pairing properties,[3e–h] while analogues derived from oxazole,[3l] imidazolidine,[3o] or 1,2,4-oxadiazole[3q] were evaluated for their inhibitory potency towards some specific enzymes. The 1,2,4-oxadiazol-3-yl analogues showed inhibitory activity towards procollagen C-proteinase,[3q] whereas the imidazo-[1,2-*a*]pyridin-6-yl[4] analogues were active as reversible inhibitors of H^+/K^+ ATPase.

Herein, we report the synthesis of novel Ψ aza′-analogues with a 5,5-bis(hydroxymethyl)-1-pyrrolin-2-yl 1-oxide residue as the glycone mimic, i.e. compounds **1** and **2** (Scheme 1). These compounds possess a nitrone function that, in addition to the uracil moiety, may act as the biologically active site. Our interest in synthesizing these compounds was motivated by their possible application as novel free radical traps. Replacement of the ribose moiety by 1-pyrroline 1-oxide, a structural unit present in a number of efficient free radical traps,[5] would be expected to have an effect on the aforementioned anti-mutagenic properties of pseudouridine.[2] Furthermore, the additional hydroxymethyl group at the 5 position of the 1-pyrroline 1-oxide ring is expected to prevent the formation of final nitroxide-free radicals in the form of different stereoisomers, a potential complication in their spectroscopic studies resulting from the presence of asymmetric carbon centres in alternative nitroxide-free radicals with one hydroxymethyl group at this position.[6] Our research on the synthesis of



Scheme 1. Retrosynthesis of the Ψ-uridine analogues **1** and **2**.

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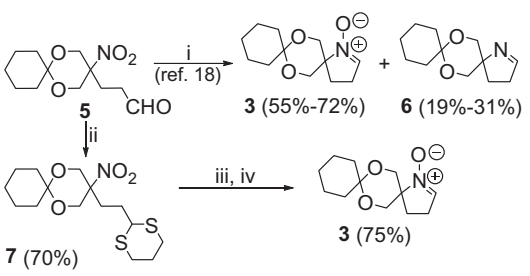
compounds **1** and **2** was also stimulated by reports on the therapeutic potential[7] of nitrones against diseases resulting from an imbalance between free radical formation and antioxidant production in the body, e.g. neurodegenerative diseases or cancer.[8] The literature revealed that the stable nitroxide-free radicals formed in this way protect cells against oxidative damage.[9] Syntheses and pharmacological properties of a number of nitrones with a heterocyclic substituent on the carbon atom of the nitrone function have been described.[5a,10] However, only a few examples of nitrones bearing a nucleobase moiety,[3l,m,11] or a nucleoside residue[12] have been reported. Recently, 5,5-dimethyl-2-(2′-deoxyuridin-5-yl)-1-pyrroline 1-oxide was reported, and postulated as a product of trapping of the 2′-deoxyuridin-5-yl radical derived from 5-halo-2′-deoxyuridines by 5,5-dimethyl-1-pyrroline 1-oxide (DMPO).[13] It was detected by HPLC/ESI-MS/MS and characterized by ^{1}H NMR spectroscopy. However, as reported in the original paper, it was not obtained on a preparative scale.

It is worth noting that nucleoside-derived free radicals (such as the 1′,2′-didehydro-2′-deoxyuridin-1′-yl radical) have attracted attention because of their postulated involvement in a variety of nucleic acid damage processes.[14]

As mentioned previously, the literature data on nitrones with a uracil-5-yl residue is rather limited.[3l,3m,13] The reported *N*-(uracil-5-ylmethylene)methanamine *N*-oxide or *N*-(uracil-5-ylmethylene)benzylamine *N*-oxide was obtained from the addition of *N*-methyl- or *N*-benzylhydroxylamine, respectively, to uracil-5-carboxaldehyde.[3l,m] Our synthetic approach to **1** and **2** (Scheme 1) involves the addition of (2,4-dimethoxypyrimidin-5-yl)lithium (**4a**) or (2,4-dimethoxypyrimidin-5-yl)magnesium bromide (**4b**) to nitrone **3**, as the key step of the synthesis. Although heteroarylation of acyclic aldonitrones with lithiated heteroaryl compounds is of great importance in the synthesis of natural or biologically active compounds,[15] the literature data on the heteroarylation of 1-pyrroline 1-oxides is rather limited; heteroarylations with 3-lithiopyridine,[16a] 2-lithiothiazole,[16b] or 2-lithiofuran[16c] have been reported. To the best of our knowledge, **4a** and **4b**, or their *O*-alkylated counterparts, have not been examined in heteroarylations of acyclic or cyclic aldonitrones. Compound **4a**, or its *O*-alkylated counterparts, were employed in the syntheses of Ψ-uridine, its stereoisomers, or pyrrolidine analogues of Ψ-uridine.[3a–c,17]

Previously, compound **3** was prepared in this laboratory from γ-nitroaldehyde **5** under reductive conditions [Zn/AcOH/Py, Scheme 2, step (i)].[18] However, the formation of **6** as a by-product was also observed. The yield of **6** depended on the zinc dust source and varied from 19% to 31%. Isolation of the organic reaction products from colloidal zinc salts, and the subsequent separation of **3** from **6** by crystallization or column chromatography were difficult. Consequently, the yields of **3** were irreproducible and varied from 55% to 72%. The most recent reports on the preparation of 1-pyrroline 1-oxides reveal that the reduction of γ-nitroaldehydes with Zn still remains one of the most common methods for their preparation.[6,19,20] However, the yields of the final nitrones depend on the reaction conditions (and probably on the specific structure of the starting γ-nitroaldehydes): 82% ($Zn/HOAc/EtOH/H_2O$)[19a] or 9–50% ($MeOH/H_2O/NH_4Cl$).[6,19b,c] Additionally, purification of the target nitrones is rather complex. Based on reports on the synthesis of 5-(diethoxyphosphoryl)-5-methyl-1-pyrroline 1-oxide[21] (or its deuterated analogues[19a]) by oxidation of the pyrrolidine precursor (39–83%, depending on the oxidant used), we envisaged the preparation of **3** via a reaction sequence involving reductive cyclisation of **5**, followed by oxidation of the pyrrolidine intermediate. However, our preliminary trials revealed that protection of the formyl group was required prior to reduction of the nitro function to an amine. Thus we anticipated that the preparation of the starting pyrrolidine would require two additional steps, i.e. the aforementioned protection of the formyl group and then its deprotection after reduction of the nitro group. It was clear that the final yield of **3** using the envisaged method would be insufficient for preparative purposes. Therefore, in order to improve the preparation of **3**, we developed an alternative procedure which involved (Scheme 2): (a) conversion of **5** into thioacetal **7**[22] by the use of $HS(CH_2)_3SH/BF_3{\cdot}Et_2O$;[23] and (b) treatment of **7** with Al/Hg in a THF/water mixture, followed by treatment of the crude reaction product with $NaHCO_3$/MeI.[24] This procedure gave **3** in a total yield of 52%. From a preparative point of view, and compared to the reference method (i), this procedure gave reproducible results and avoided the difficult separation of **3** from the reaction mixture.



Scheme 2. Reagents and conditions: (i) Zn, AcOH, Py, EtOH, −5–3 °C, 3 h; (ii) $HS(CH_2)_3SH$, $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C to rt, 2 h; (iii) Al/$HgCl_2$, THF, H_2O, rt, 2 h; (iv) $NaHCO_3$, MeI, MeCN, H_2O, 45 °C, 2 h.

Next, the reaction of **3** with (2,4-dimethoxypyrimidin-5-yl)lithium (**4a**) was examined (Scheme 3). Formation of **4a** from **8** or 5-iodo-2,4-dimethoxypyrimidine by the action of *n*-BuLi[3b,c,17a,25] or *t*-BuLi,[17g] has been reported. In light of the reports on the addition of *n*-BuLi to ketonitrones[26] (less active acceptors than aldonitrones), we decided to use *t*-BuLi to form **4a**. In contrast to *n*-BuLi, *t*-BuLi did not undergo the addition under the reported conditions.[16a] Our procedure involved: treatment of **8** (1 equiv) with *t*-BuLi [1.2 equiv, variant (ia); or 2 equiv, variant (ib)] at −78 °C; (ii) addition of **3** at −78 °C and stirring the reaction mixture at room temperature for 3 h, followed by removal of the volatiles; and (iii) treatment of the residue with $Cu(OAc)_2/NH_3$ (aq) at room temperature.[27] Variant (a) of this procedure, i.e. when **8** was treated with 1.2 equiv of *t*-BuLi in order to produce **4a**, gave 2,2′-binitrone **9** (34%) and **10** (71%). Variant (b), i.e. when **8** was reacted with 2 equiv of *t*-BuLi, afforded the desired compound **11** (17%) accompanied by **9** (39%) and **10** (70%).[28] The starting compound **3** was not recovered from the reaction mixtures. These results suggest that, in both variants of this reaction sequence, dimerisation of **3** predominated over the addition of **4a** to **3**. Presumably, the dimerisation was initiated by deprotonation of **3** at the 2 position by *t*-BuLi or **4a** (Scheme 3c). Addition of the resulting **3-Li** to **3**, followed by hydrolysis of **9-Li** and the subsequent oxidation furnished **9**. Dimer **9** was also obtained (31%) by the treatment of **3** (2 equiv) with *t*-BuLi (1 equiv) under the same conditions. In a separate experiment, when the reaction was quenched with wet Et_2O at −50 °C, prior to the oxidation step, TLC ($CHCl_3$/acetone, 85/15, v/v) showed the presence of both **3** and **9** in the reaction mixture; the mixture was not separated, and the ratio of **3**/**9** was not determined. These findings suggest that the reaction temperature was not a decisive factor in the formation of **9**. Such dimerisation of 1-pyrroline 1-oxides upon treatment with NaH (liquid NH_3), *n*-BuLi (−70 °C) or LDA (−70 °C) has been reported.[29] The literature revealed that the high reactivity of cyclic aldonitrones towards metalation at the nitrone carbon atom is a consequence of their fixed (*E*)-configuration. Stabilization of the organolithium carbanion (such as **3-Li**) by intramolecular coordination of lithium with the nitrone oxygen is postulated as a factor facilitating the metalation.[30] To the best our knowledge, competition between addition of an "external" nucleophile to an aldonitrone and dimerisation