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Novel intramolecular aminohydroxylation toward the syntheses of 2′-amino-2′-ethynyl nucleosides

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ABSTRACT

Syntheses of both 2′-amino-2′-ethynyl guanosine and uridine, using an intramolecular aminohydroxylation reaction as the key step, are described. The corresponding 5′-*O*-triphosphates of the aforementioned nucleosides were obtained and the inhibitory activity was subsequently evaluated against the hepatitis C virus NS5B polymerase.

Nucleosides, the monomeric building blocks of DNA and RNA, characteristically consist of base and ribose subunits. Chemically modified unnatural nucleoside derivatives that disrupt DNA and RNA assembly have delivered drugs for decades that combat cancer and a variety of viral diseases [1]. Despite a long history of success, the need for additional nucleoside antiviral agents has never been greater. This has been emphasized most recently with the outbreak of the COVID-19 pandemic and the rapid identification of the modified nucleosides Remdesivir (**1**, Fig. 1**)** for immediate development [2].

As part of a medicinal chemistry effort toward anti-HCV agents, we previously reported that 5′-O-triphosphates derived from nucleosides bearing C2′-α-amino [3] and C2′-β-ethynyl [4] (**3** and **4**; Fig. 2) functionalities proved to be effective inhibitors of the NS5B polymerase. In fact, compound **4** demonstrated superior *in-vitro* potency compared to its C2′-β-methyl analogue [5] and displayed an order of magnitude IC_{50} improvement over the triphosphate of the nucleoside contained within the pro-drug blockbuster Sofosbuvir (**2;** Fig. 1) in the same assay [4–6].

As we searched for novel antiviral analogs with improved activity and potentially better physicochemical properties, a logical progression of this work involved incorporating both the amino and ethynyl groups at the 2′ position. Herein, we describe the syntheses and biological evaluation of the uridine **5** and guanosine derivatives **6** (Fig. 3).

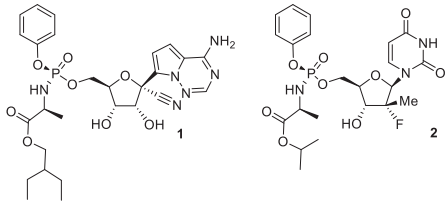

Fig. 1. Remdesivir (1) and Sofosbuvir (2).

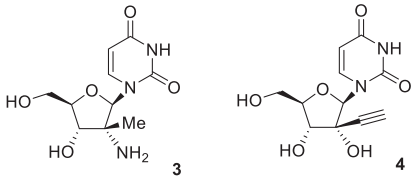

Fig. 2. C2′-α-amino and C2′-β-ethynylnucleosides.

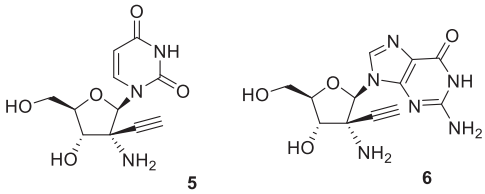

Fig. 3. C2′-α-amino 2′-ethynyl uridine and guanosine.

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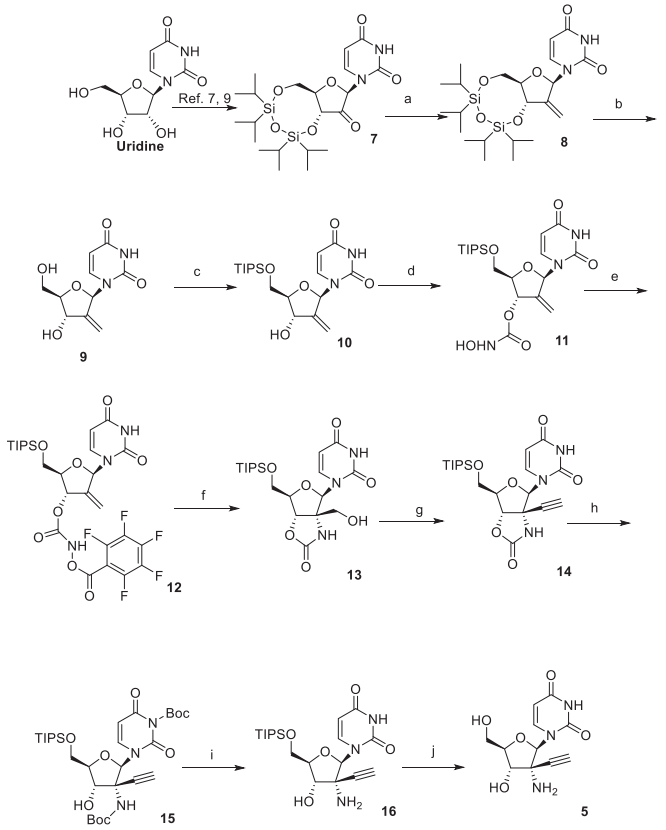
0040-4039/

At first sight, the synthetic incorporation of a tertiary amino group at the 2′-position in a nucleoside would appear to be a daunting task. Our synthesis of compound **3** involved a two-step process: diastereoselective cobalt catalyzed hydroazidation to the less sterically encumbered α-face of a nucleoside containing 2′-exocyclic methylene and subsequent catalytic hydrogenation to deliver the desired functionality [7]. A novel synthetic approach was required to incorporate an ethynyl group on the β-face of the ribose. To this end, the synthesis of the uridine analogue **5**, involving a diastereospecific aminohydroxylation reaction as the key step**,** is outlined in Scheme 1 [8]**.**

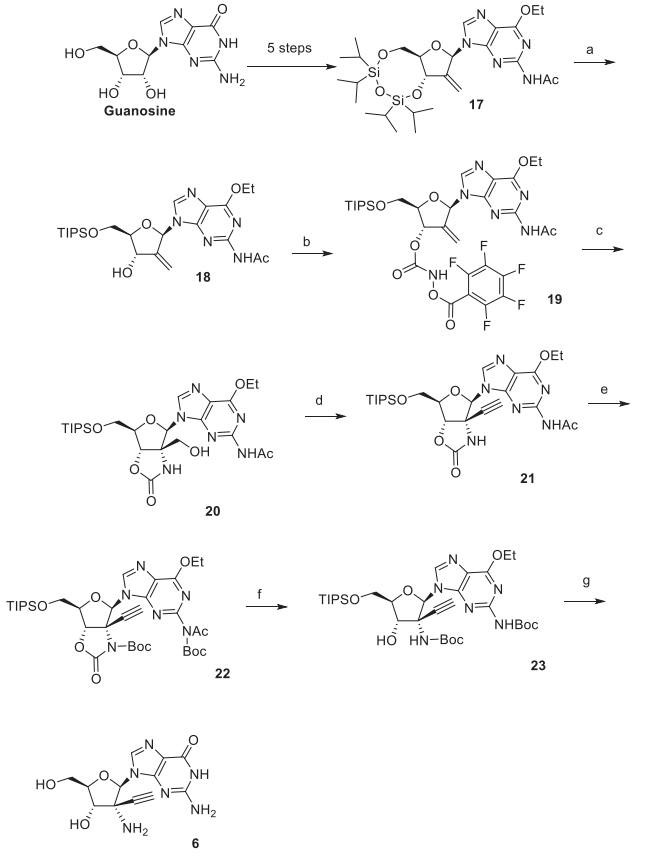
The ketone **7** was prepared, as previously described, in two steps, from the readily available parent nucleoside [7,9]. Subsequent Wittig olefination provided the neccessary exocylic olefin **8**, in moderate yield. The disiloxane protecting group was removed with fluoride and the intermediate diol **9** was selectively reprotected with triisopropylsilyl triflate, leaving the 3′-hydroxyl exposed for further transformation. When treated with CDI followed by hydroxylamine, the secondary alcohol **10** generated the *N*-hydroxy carbamate **11**. Acylation with pentafluorobenzoyl chloride provided the aroyloxycarbamate **12**, following silica gel column chromatography to remove the bis-acylated by-product. Having set the stage for the key process, Donohoe's modified tethered amino-hydroxylation successfully delivered the desired cyclic carbamate **13** in 52–60% isolated yield. Dess-Martin periodinane oxidation [10] and exposure of the resulting crude aldehyde with the Ohira-Bestmann reagent [11] installed the β-ethynyl group of the masked nucleoside **14**. When treated with excess di-*tert*-butyl dicarbonate, the oxazolidinone **14** generated the desired bis-acylated adduct, which was selectively methanolysed to the alcohol **15** with Cesium carbonate. Removal of the Boc groups in **15** was achieved with TFA, giving the free amine **16** on treatment of the salt with excess triethylamine. Finally, fluoride removal of the silyl ether revealed the desired nucleoside **5** [12].

Following a similar strategy, the synthesis of the guanosine derivative **6** is outlined in Scheme 2.

The olefin **17** was synthesised in gram quantities from guanosine in 5 steps [7]. As described previously, the intermediate diol was generated from the bis-silyl protected **17** and the 5′-OH was selectively masked using TIPSOTf to give the exposed 3′-alcohol **18**. Notably, attempts toward the same transformation using the relatively less reactive silylating reagent TIPSCl failed to generate any desired product. The silyl ether **18** was transformed to the pentafluoroester **19** *via* the *N*-hydroxycarbamate without incident. Continuing in parallel with the uridine example in Scheme 1, the tethered amino-hydroxylation proceeded to the oxazolidinone **20** in a similar 50% isolated yield. Subsequent Dess-Martin oxidation and treatment of the resulting aldehyde with the Ohira-Bestmann reagent provided the desired alkyne in an excellent combined yield (88%). To simultaneously activate the acetamide and oxazolidinone to methanolysis and initiate systematic removal of the protecting groups, the masked nucleoside **21** was exposed to di-*tert*-butyldicarbonate to provide the bis-boc adduct **22.** Subsequent methanolysis of both the oxazolidinone and amide functionalities



Scheme 1. Reagents and conditions. a) Ph_3PMeBr, KHMDS, (52%); b) TBAF, THF, 25 °C (87%); c) TIPSOTf, Pyridine/2,6-Lutidine, 0 °C (76%); d) CDI, imidazole followed NH_2OH:HCl, Pyridine, 25 °C, (73%); e) Pentafluorobenzoyl chloride, Et_3N, CH_2Cl_2, −20 °C (90%); f) $K_2OsO_2(OH)_4$, nPrOH/H_2O 25 °C, (52–60%); g) Dess-Martin periodinane, CH_2Cl_2, 25 °C (used crude) followed by Ohira-Bestmann reagent, K_2CO_3, MeOH, 25 °C (88% over two steps); h) Boc_2O, DMAP, CH_2Cl_2, 25 °C followed by Cs_2CO_3, MeOH, 25 °C (71%); i) TFA, CH_2Cl_2, 25 °C followed by Et_3N, 90% j) TBAF, THF, 25 °C (83%).



Scheme 2. Reagents and conditions. a) i) TBAF, THF, 25 °C (85%); ii) TIPSOTf, Pyridine/2,6-Lutidine, 0 °C (79%); b) i) CDI, imidazole, NH_2OH:HCl, pyridine, 25 °C (58%); ii) Pentafluorobenzoyl chloride, Et_3N, CH_2Cl_2, 0 °C (91%); c) $K_2OsO_2(OH)_4$, nPrOH/H_2O 25 °C, (50%); d) Dess-Martin periodinane, CH_2Cl_2, 25 °C (used crude) followed by Ohira-Bestmann reagent, K_2CO_3, MeOH, 25 °C (88% over two steps); e) Boc_2O, DMAP, CH_2Cl_2, 25 °C (86%); f) Cs_2CO_3, MeOH, 25 °C (71%); g) 1 N HCl (aq), THF, 55 °C followed by Et_3N, (55%).

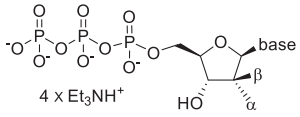
to bis-carbamate **23** was accomplished with cesium carbonate at room temperature. The silyl ether, ethyl enolether, and *tert*-butyl carbamates were cleaved simultaneously in one pot using warm dilute aqueous HCl in THF over the course of two days, revealing the desired nucleoside **6** [13].

The initial phosphorylation during the metabolic step-wise transformation of a nucleoside to its NTP can be rate-limiting as observed with 2′-F guanosine analogs, and measurements of cell-based activity may not accurately reflect the intrinsic potency of the active NTP metabolite [14]. To ascertain their effectiveness as potential antiviral agents both nucleosides **5** and **6** were converted to the corresponding 5′-O-triphosphates **24a** and **25a** [15]. Their inhibitory activity against wild-type HCV NS5B polymerase was evaluated along with other relevant nucleoside triphosphates (Table 1) [16].

A direct comparison of the β-ethynyl **24a** with its methyl counterpart **(24c)** revealed a modest 3–4 fold boost in potency in the uridine nucleoside series. This effect was more pronounced with a hydroxyl group in the C2′-α position (**24b** vs. **24d**) [4]. In the guanosine example, this effect was reversed, the acetylene **25a** being approximately 2-fold less potent than the known methyl analogue **25b**. Importantly, both novel nucleoside triphosphates **24a** and **25a** exhibit comparable potency with the triphosphate derived from Sofosbuvir **24e** and clearly merit further consideration.

In summary, we have synthesized biologically relevant nucleosides bearing an ethynyl at the C2′- β-position and an amino as the α-moiety from inexpensive and readily available uridine and guanosine. Our route highlighted a key synthetic transformation, in which a tethered aminohydroxylation was employed to prepare a highly functionalized ribose core with diastereospecificity. To evaluate their potential as anti-HCV agents, the corresponding 5′-*O*-triphosphates were prepared and their inhibitory activity against wild type NS5B polymerase was measured. While the ethynyl amino uridine derivative **24a** was approximately 3 times more potent that the C2′- β-Me analogue **24c**, the introduction of the ethynyl group into guanosine **25a** had the opposite effect. Ultimately, both triphosphates have IC_{50}'s in the low single digit μM range, comparable to the metabolite **24e** formed on administration of Sofosbuvir. Application of this and other studies related to the nucleosides described in this paper will be presented elsewhere.

Table 1
Activities of the nucleoside triphosphates against NS5B polymerase.



base = Uridine **24** or Guanosine **25**

NTP	α	β	IC_{50} (μM)
24a	—NH_2	—≡	3.2
24b	—OH	—≡	0.09
24c	—NH_2	—Me	11
24d	—OH	—Me	0.43 [a]
24e	—NH_2	—≡	1.1 [b],[14]
25a	—NH_2	—Me	1.3
25b	—F	—Me	0.6

[a] Prepared and tested as dimethylhexylammonium salts
[b] Prepared and tested as sodium salts.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tetlet.2021.153066.

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[12] MS and NMR for Compound 5. 1H NMR (599 MHz, CD3OD): δ8.10(d; J = 8.12 Hz; 1H); 5.90 (s; 1H); 5.67 (d; J = 8.11 Hz; 1H); 4.26 (d; J = 8.22 Hz; 1H); 3.93-3.94 (m; 2H); 3.75 (dd; J = 12.32; 2.93 Hz; 1H); 3.22 (t; J = 8.46 Hz; 1H); 2.92 (s; 1H). 13C NMR (151 MHz, CD3OD): δ166.28,152.72,143.95,142.50,136.17, 120.99,108.72,102.23,92.70,84.86,84.48,75.97,61.81,60.75,59.66,59.64,59.64, 59.62,24.93,20.88, 20.87, 14.09, [M+1]=268.27.
[13] MS and NMR for Compound 6. 1H NMR (599 MHz, CD3OD) δ 8.16 (s, 1H), 5.93 (s, 1H), 4.53 (d, J = 7.9 Hz, 1H), 4.06 (dt, J = 7.9, 3.2 Hz, 1H), 3.96 (dd, J = 12.4, 2.8 Hz, 1H), 3.82 (dd, J = 12.4, 3.6 Hz, 1H), 2.70 (s, 1H). 13C NMR (151 MHz, CD3OD) δ 159.46, 155.49, 153.02, 138.05, 117.49, 91.70, 84.98, 84.27, 76.01, 75.69, 62.36, 61.46.[M+1]=307.17.
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[15] General Preparation of Nucleoside 5'-Triphosphates The preparation 5'-triphosphates.(24a-c and 25a-b) were carried under contractual agreement with TriLink biotechnologies, San Diego, CA.and analysed as the triethylammonium salts. Triphosphates 24d and 24e were prepared in a similar manner and examined as the dimethylhexylammonium salts and sodium salts, respectively. For example: A solution of the nucleoside (0.05 mmol) in trimethylphosphate (1 ml) was placed under an atmosphere of nitrogen. To this solution was added proton sponge (17 mg; 0.08 mmol) and the resulting mixture was cooled in an ice bath. Phosphoryl trichloride (32 mg;

0.21 mmol) was added and stirring was continued for 4h. Pyrophosphate (200 mg; 0.21 mmol), DMF (1 ml) and tributylamine (0.03 ml) were added and stirring was continued for a further 1h. The reaction was quenched by the addition of triethylammonium bicarbonate buffer (1.0M; 3ml) and the volatiles removed under reduced pressure. The residue was purified by HPLC using: (1 #-Pre-HPLC-001 (SHIMADZU)): Column, 1 #-PrepC-008 (Atlantis HILIC silica 19*150 186003959 0110182551 kk 03), using acetonitrile and water with ammonium bicarbonate (50mmol); Detector, UV 220 & 254 nm. 24a, MS (ESI) m/z 507.0 [M]; 24b, MS (ESI) m/z 507.0 [M]; 24c, MS (ESI) m/z 496.8 [M]; 24e, MS (ESI) m/z 499.1 [M]; 25b, MS (ESI) m/z 535.8 [M].

[16] Inhibition of HCV NS5B Polymerase by Nucleoside Triphosphate Analogs. This assay is a modified version of the assay described in International Publication No. WO2002/057287. Briefly, 50 μL reactions containing 20 mM HEPES (pH 7.3); 7.5 mM DTT; 20 units/ml RNasIN; 1 μM each of ATP, GTP, UTP and CTP; 20 μCi/mL [33P]-CTP; 10 mM MgCl; 60 mM NaCl; 100 μg/ml BSA; 0.021 μM DCoH heteropolymer RNA template; and 5 nM NS5B (1b-BKΔ55) enzyme are incubated at room temperature for 1 hour. The assay is then terminated by the addition of 500 mM EDTA (50 μL). The reaction mixture is transferred to a Millipore DE81 filter plate and the incorporation of labeled CTP is determined using Packard TopCount. Compound IC50 values can then be calculated from experiments with 10 serial 3-fold dilutions of the inhibitor in duplicate. The intrinsic potency (Ki) of an NTP inhibitor is derived from its NS5B IC50 using the Cheng-Prusoff equation for a competitive inhibitor, as described in Cheng et al., Biochem Pharmacol 1973, 22, 3099-3108: Ki = IC50 / (1+[S]/Km), where [S] = 1 μM, and Km is the concentration of cognate NTP yielding half-maximal enzyme activity in the assay absent exogenous inhibitors.