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Synthesis and antiviral evaluation of 4′-(1,2,3-triazol-1-yl)thymidines†

Sanjeev Kumar V. Vernekar, Li Qiu, Jeana Zacharias, Robert J. Geraghty and Zhengqiang Wang*

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Non-obligate chain terminating nucleosides with a linear substituent (azido or ethynyl group) at the 4′ position represent an important class of compounds in antiviral discovery, particularly against hepatitis C virus (HCV) and human immunodeficiency virus (HIV). We have previously shown that 3′-azidothymidine (AZT)-derived 1,2,3-triazoles can be potent inhibitors of HIV-1. To gauge the medicinal chemistry impact of functionalizing the 4′-linear substituent and possibly generate novel antiviral nucleoside scaffolds, we have explored azide–alkyne cycloaddition reactions with 4′-azidothymidine (ADRT). The Ru-mediated reaction failed and the Cu-catalyzed variant generated 1,2,3-triazoles (**9a–y**) with only modest yields and efficiencies, indicating a substantial steric barrier around the 4′-azido group. Antiviral screening identified a few triazole analogues moderately active against HIV-1 (18–62% inhibition at 10 μM) and/or influenza A virus (15–50% inhibition at 10 μM), and none active against West Nile virus (WNV) or HCV. These results suggest that the linear 4′ azido group of ADRT may be essential for target binding and that its chemical manipulation could largely compromise antiviral potency.

Introduction

Synthetic nucleoside analogues provide an important platform for drug discovery, especially as anticancer and antiviral agents.[1] By mimicking endogenous nucleosides, these analogues exploit cellular kinases for phosphorylation and compete against nucleoside triphosphates (NTPs) or deoxynucleoside triphosphates (dNTPs) for active site binding and incorporation during RNA or DNA polymerization. Mechanistically, nucleoside analogues could act as obligate chain terminators when lacking the 3′-OH group,[2] as exemplified by numerous nucleoside reverse transcriptase inhibitors (NRTIs) for the treatment of HIV infection.[3] The absence of the 3′-OH, however, could reduce affinities for cellular kinases and viral polymerases, and ultimately lead to suboptimal antiviral efficacies.[4] Alternatively, incorporated analogues bearing the 3′-OH group could also terminate the growing RNA or DNA chain when chemical modifications at the 2′ or 4′ position sterically hinder the proper alignment of the 3′-OH with the incoming NTP or dNTP.[2] By virtue of the 3′-OH group, these non-obligate chain terminators presumably benefit binding to both kinases and polymerases. This critical advantage has been successfully explored in the discovery of novel antivirals, particularly against HCV, as major nucleoside scaffolds of HCV NS5B polymerase inhibitors, such as the Idenix NM107 (**1**),[5] the Pharmasset PSI-6130 (**2**)[6,7] and the Roche R1479 (**3**),[8,9] all have the 3′-OH group (Fig. 1).[10]

HIV NRTIs bearing the 3-OH group are rare, with the notable exception of two 4′ modified deoxynucleosides: 4′-ethynyl-2-fluoro-2′-deoxyadenosine (EFdA, **4**), an exceptionally potent clinical candidate with an EC_{50} of 0.05 nM against HIV-1 in cell culture;[11] and ADRT (**5**)[12,13] with an antiviral potency comparable to that of AZT (**6**, Fig. 2). Mechanistically EFdA terminates the chain *via* stabilizing RT at the N site to cause translocation deficiency[14] and ADRT retains its activity against AZT-resistant HIV mutants,[13] suggesting a mechanism of RT inhibition distinctive of typical NRTIs. Interestingly, both the non-obligate chain terminating NRTIs **4–5** feature a linear 4′ modifier (ethynyl and azido) which can be important for their unique mechanism of inhibition. Chemical manipulation of these linear functional groups into new chemical moieties of completely different shapes and volumes might lead to novel nucleoside scaffolds with distinct binding profiles to both kinases and polymerases. Recently, we have demonstrated for the first time that AZT can be clicked into 1,2,3-triazoles with potent anti-HIV activities and interesting resistance profiles (Fig. 2a).[15] Given the importance of the 4′-modifier in the discovery of non-obligate chain terminating nucleoside antivirals, we were prompted to extend our clicking efforts to ADRT with the hope to generate 4′-triazole antiviral scaffolds (Fig. 2b). To the best of our knowledge synthetic or biological studies of such 4′-triazole nucleosides are currently unknown. Unlike the

Center for Drug Design, Academic Health Center, University of Minnesota, Minneapolis, MN 55455, USA. E-mail: wangx472@umn.edu; Fax: +1 612 625-8154; Tel: +1 612 626-7025

† Electronic supplementary information (ESI) available: Chemistry, experimental details, and full characterization data (^{1}H NMR, ^{13}C NMR and HRMS) for newly synthesized compounds; protocols for assays against HIV, influenza virus, WNV and HCV. See DOI: 10.1039/c4md00039k

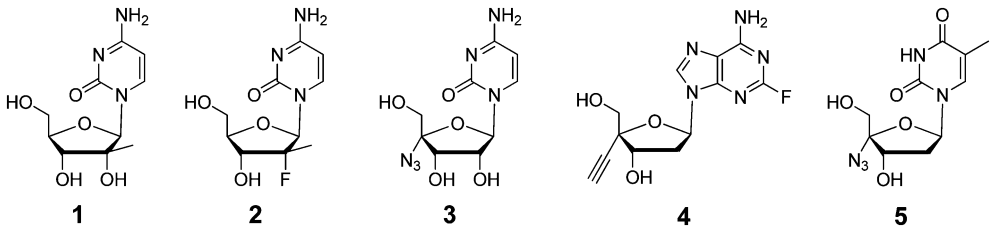


Fig. 1 Examples of non-obligate chain terminating antivirals. NM107 (**1**) and PSI-6130 (**2**) achieve chain termination through 2′ modification while R1479 (**3**), EFdA (**4**) and ADRT (**5**) act through 4′ substitution. **1–3** are HCV NS5B polymerase inhibitors and **4–5** are HIV NRTIs.

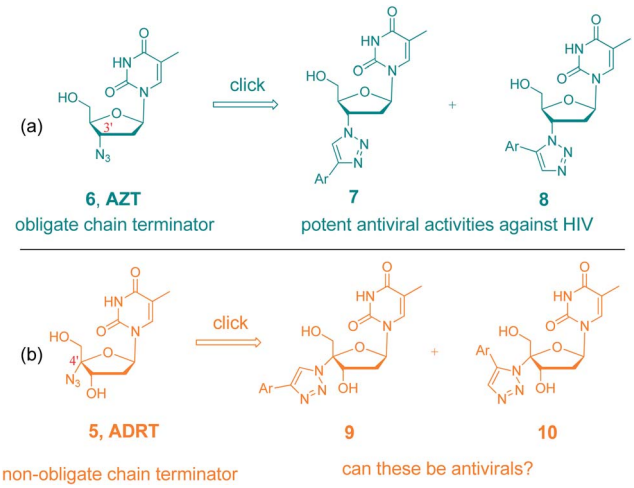


Fig. 2 Click azido nucleoside analogues into novel nucleoside antiviral scaffolds. (a) Clicking obligate chain terminator AZT generates novel 1,2,3-triazole nucleoside analogues active against HIV. (b) Can non-obligate chain terminator **5** be clicked into novel antiviral scaffolds?

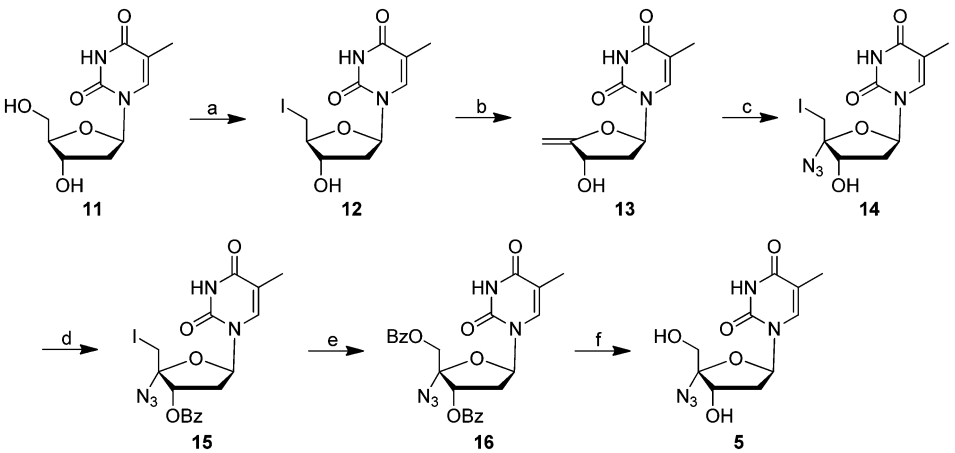
click chemistries with AZT where both 1,4 (**7**) and 1,5 (**8**) disubstituted triazole nucleoside scaffolds can be easily accessed,[15] clicking of ADRT proved viable only *via* the 1,4 path to yield scaffold **9**. We report herein the synthesis and antiviral evaluation of disubstituted 4′-(1,2,3-triazol-1-yl)thymidines (**9**).

Results and discussion

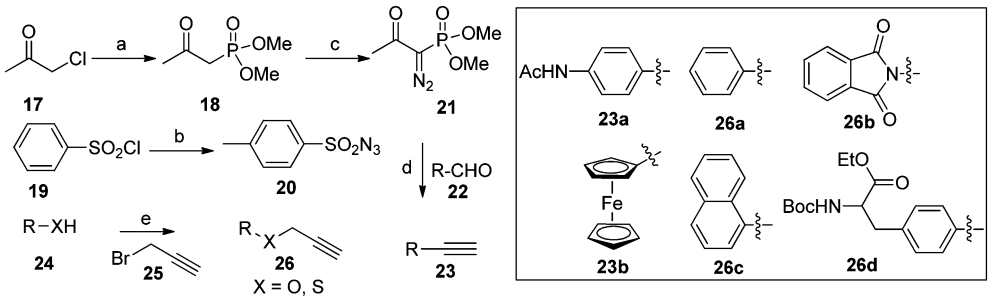
Chemistry

ADRT (**5**) was synthesized from thymidine (**1**) following the literature method[16] as outlined in Scheme 1. The synthesis featured an exocyclic alkene intermediate **13** which was prepared through an E2 reaction of an iodide precursor **12** according to the procedure reported by Verheyden and Moffatt.[16] The iodide precursor **12** was readily prepared from thymidine with I_2 and PPh_3 in good yields. The key step for the synthesis was the preparation of azide **14** in a stereoselective fashion. This was achieved in moderate yield by treating **13** with benzyltriethylammonium chloride, sodium azide and iodine to afford a diastereomeric mixture of **14** (de = 90%), which can be separated by careful chromatography. The desired isomer was then benzoylated at the 3′-hydroxy group, followed by an oxidative displacement of the 5′-iodo group with *m*-CPBA and *m*-chlorobenzoic acid to afford a dibenzoylated intermediate **16**. Finally, removal of the benzoyl protecting groups with ammonia in MeOH delivered **5** in good yield. The NMR data of **5** were fully consistent with the literature.[13]

The aliphatic and aromatic alkynes (**23**) were either commercially available or prepared from aldehydes *via* the Seyferth–Gilbert homologation with the Bestmann reagent[17] **21** in good yields (Scheme 2). The propargyl aryl ethers or thiol ethers (**26**) were readily prepared *via* alkylation of phenols or thiophenols with propargyl bromide and K_2CO_3 in good yields (Scheme 2).[18]



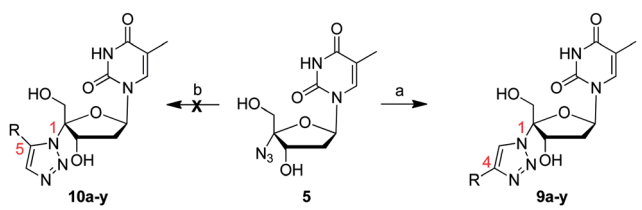
Scheme 1 Synthesis of ADRT **5**. Reagents and conditions: (a) I_2, PPh_3, imidazole, THF, 15–25 °C, 18 h, 77%; (b) NaOMe, MeOH, 60 °C, 15 h, 81%; (c) $[Bn(Et)_3N]Cl$, NaN_3, NMO, I_2, THF, 0–5 °C, 2 h, 52%; (d) BzCl, DMAP, pyridine, rt, 3 h, 81%; (e) $(Bu_4N)HSO_4$, K_2HPO_4, *m*-chlorobenzoic acid, *m*-CPBA, DCM–H_2O (4 : 1), rt, 15 h, 84%; and (f) 7 N NH_3–MeOH, rt, 12 h, 79%.



Scheme 2 Synthesis of alkynes **23** and **26**. Reagents and conditions: (a) KI, $P(OMe)_3$, acetone–CH_3CN, rt–50 °C, 12 h, 72%; (b) NaN_3, acetone–H_2O, 0 °C–rt, 14 h, 94%; (c) NaH, **20**, THF–benzene, 0 °C–rt, 12 h, 65%; (d) K_2CO_3, MeOH, rt, 12 h, 45–90%; and (e) propargyl bromide, K_2CO_3, DMF, rt, 12–15 h, 60–76%.

The preparation of alkynes **23** and **26** allowed us to functionalize the azido group of ADRT (**5**) through click chemistry. It is well established that the Huisgen cycloaddition between an azide and an alkyne can be effected regioselectively through two distinct catalytic pathways: the copper(I)-catalyzed azide–alkyne cycloaddition (CuAAC)[19] generates exclusively 1,4-disubstituted 1,2,3-triazoles, while the ruthenium(II)-catalyzed variant (RuAAC)[20] produces 1,5-disubstituted 1,2,3-triazoles. The combination of CuAAC and RuAAC enables the preparation of triazoles with diverse residues and substitution patterns (1,4 *vs.* 1,5). In our case, the CuAAC reaction of ADRT (**5**) with alkynes (**23a,b** or **26a–d**) produced 4′-(1,2,3-triazol-1-yl)thymidines (**9a–y**) in moderate yields (Scheme 3), whereas the RuAAC variant failed and the expected 1,5-disubstituted triazoles **10** were not observed (Scheme 3). Further investigation of reaction conditions, including solvent effect, catalyst, co-catalyst, temperature, method of heating (microwave *vs.* conventional), yielded no significant improvement. These unsuccessful attempts strongly suggest that nucleoside tertiary azides are essentially non-clickable *via* the RuAAC pathway, presumably due to steric hindrance around the azido group. The steric barrier for clicking tertiary azide **5** is further manifested through the Cu-mediated click reaction, which proceeded with a substantially lower efficiency than the same reaction with secondary azide AZT (**6**). With ADRT (**5**) the completion of the CuAAC reaction required prolonged time (typically 5–7 days), elevated temperature (60 °C) and intermittent addition of a fresh catalyst (every 24 h). Not surprisingly, the size of alkyne substrates also appeared to impact the reaction as aromatic alkynes required elongated time when compared to alkynyl aryl ethers, though the overall success of the CuAAC reaction depends more on the steric nature of the azide partner. The low efficiency of cycloadditions with tertiary azides is known.[21]

It must be pointed out that incomplete consumption of **5** will render chromatographic separation extremely tedious as triazole products tend to have very similar Rf to ADRT. Caution has to be taken to avoid any ADRT contamination which will likely cause false positive in the biological assays. Purity of the final 4′-(1,2,3-triazol-1-yl)thymidines (**9a–y**) was confirmed by NMR and through HPLC analysis.

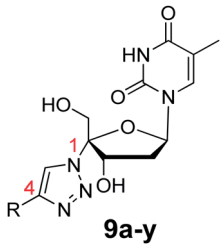


Scheme 3 Clicking ADRT **5**. Reagents and conditions: (a) **23** or **26**, sodium ascorbate, $CuSO_4 \cdot 5H_2O$, $THF–H_2O$ (3 : 1), 60 °C, 5–7 days, 33–72% and (b) **23** or **26**, $Cp^*RuCl(PPh_3)_2$, THF, 60 °C.

Antiviral testing

We first screened all final compounds against HIV-1 with a cytoprotection assay based on viral cytopathic effects (CPE).[22] The synthesized 4′-(1,2,3-triazol-1-yl)thymidines (**9a–y**) were evaluated against an IIIB strain of HIV-1 and the results are summarized in Table 1. While the screening identified two compounds **9d** (62%) and **9h** (60%) with significant antiviral activities and four others (**9e**, **9m**, **9p** and **9v**) with modest (18–31%) cell protection, the observation that most analogues in this series were either marginally active or completely inactive indicates a general trend of low potency and dampens our enthusiasm on further testing the two active analogues. In the meantime, parallel cell control with mock infection revealed that these 4′-triazole analogues generally do not exhibit cytotoxicity, which prompted us to conduct additional screenings against three RNA viruses: influenza A, WNV and HCV. As aforementioned, 2′-deoxy non-obligate chain terminators can be potent inhibitors of RNA viruses, *e.g.* compound **2** (ref. 6 and 7) against HCV (Fig. 1). The influenza A virus antiviral assay is a cytoprotection assay while the WNV and HCV assays use replicon-containing cells to evaluate potential antivirals. Interestingly, our initial influenza A screening assay using A549 cells identified seven compounds (**9h–k**, **9n**, **9r**, and **9y**) with apparent antiviral activities ranging from 15% to 50% at 10 μM. However, these activities were not confirmed through further testing in MDCK cells as all seven analogues were found inactive. Finally, the screening of all 4′-(1,2,3-triazol-1-yl)thymidine analogues (**9a–y**) in replicon assays yielded no compound with appreciable inhibitory activity against WNV or HCV. Altogether, these screening results suggest that, with the exception of the moderate inhibitory activities observed with a few analogues

Table 1 Antiviral screening of 4′-(1,2,3-triazol-1-yl)thymidine analogues (**9a–y**) against HIV, and influenza



9a-y

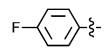
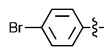
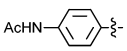
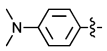
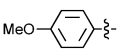
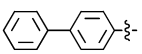
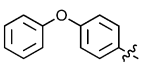
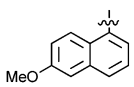
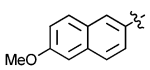
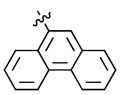
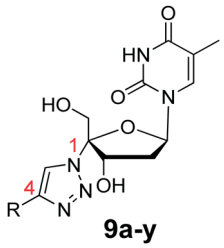
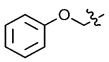
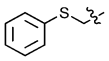
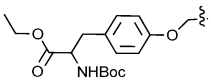
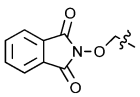
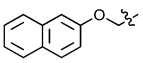
		HIV		Influenza (inhibition% @ 10 μM)	
Compd	R	Inhibition% @ 10 μM	Viability% @ 10 μM	A549 cells	MDCK cells
9a		0	99	0	NT
9b		5	100	3.1	NT
9c		0	96	2.2	NT
9d		62	94	0	NT
9e		19	100	0	NT
9f		0	99	0	NT
9g		0	95	0	NT
9h		60	100	18	4.0
9i		0	98	54	3.0
9j		12	100	21	1.0
9k		0	84	19	1.0
9l		0	100	5.0	NT
9m		31	80	1.7	NT
9n		1	95	48	1.0
9o		0	95	5.3	NT
9p		18	95	0	NT
9q		0	86	0	NT
9r		0	95	15	0
9s		2	100	0	NT

Table 1 (*Contd.*)



9a-y

Compd	R	HIV		Influenza (inhibition% @ 10 μM)	
		Inhibition% @ 10 μM	Viability% @ 10 μM	A549 cells	MDCK cells
9t		8	100	0	NT
9u		0	97	10	NT
9v		20	99	3.3	NT
9w		0	97	0	NT
9x		1	99	3.7	NT
9y		0	93	16	1.0

against HIV-1 and/or influenza A virus, ADRT-derived triazoles generally lack the potency to be considered viable antiviral scaffolds, a stark contrast to AZT-derived triazoles which showed potent inhibitory activities against HIV-1.

Conclusion

In an attempt to study the medicinal chemistry impact of functionalizing the linear 4′ substituent of non-obligate chain terminating nucleosides and possibly generate novel antiviral nucleoside scaffolds, we have conducted both CuAAC and RuAAC reactions with 4′-azido nucleoside ADRT. The observed low efficiency of these reactions reflects severe steric hindrance around the 4′-azido modifier. Antiviral screening identified a few analogues moderately active against HIV-1 (18–62% inhibition at 10 μM) and/or influenza A virus (15–50% inhibition at 10 μM), and none active against WNV or HCV. These results suggest that chemically modifying the linear 4′ modifier of non-obligate chain terminating nucleosides may not be tolerated in antiviral drug discovery.

Abbreviations

HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
AZT	3′-Azidothymidine
ADRT	4′-Azidothymidine
WNV	West Nile virus
NTP	Nucleoside triphosphate
dNTP	Deoxynucleoside triphosphate
RT	Reverse transcriptase
NRTI	Nucleoside reverse transcriptase inhibitor
EFdA	4′-Ethynyl-2-fluoro-2′-deoxyadenosine
CuAAC	Copper(I)-catalyzed azide–alkyne cycloaddition
RuAAC	Ruthenium(II)-catalyzed azide–alkyne cycloaddition
CPE	Cytopathic effect

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