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[d4U]-butyne-[HI-236] as a non-cleavable, bifunctional NRTI/NNRTI HIV-1 reverse-transcriptase inhibitor

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Abstract—The synthesis of bifunctional compound **10** consisting of d4U joined at C-5 to a butynyl spacer attached to HI-236 is reported using a Sonogashira coupling as a key step. As a non-cleavable bifunctional HIV inhibitor incorporating an NRTI with an NNRTI, **10** shows good inhibitory activity (EC_{50} = 250 nM) against HIV (IIIB) replication in MT-2 cell culture, which is eight times greater than that of d4T and between those of the two component drugs.

The use of combination therapy (HAART) for HIV is designed to prevent the development of resistant HIV strains by effectively suppressing viral replication, thus denying HIV the opportunity to produce new mutations.[1] Non-nucleoside reverse transcriptase inhibitors (NNRTIs) act allosterically by slowing down the chemical step catalysed by the HIV reverse transcriptase (RT) enzyme, and this retardation allows the two-step binding of the nucleoside analogues (NRTI) to come to equilibrium leading to tighter binding of the nucleotide.[2] These studies suggested that the nucleotide triphosphate and non-nucleoside inhibitors could simultaneously bind to their respective NRTI and NNRTI sites in RT and that there was communication between the two sites. The close proximity (10–15 Å) of the NRTI/NNRTI binding sites has inspired several groups to synthesize bifunctional heterodimers containing one of each class of drug separated by a spacer following an original suggestion by Nanni.[3a] The heterodimers (double-drugs) reported fall into two distinct classes, with the more common of the two joining the two drugs via a cleavable linker in the expectation that both drugs will be released into the cell cytoplasm, resulting in synergistic inhibitory effects. The other class involves incorporation of a non-cleavable linker in the hope of achieving concurrent inhibition at both target sites. In principle, such a bifunctional inhibitor would utilize the additive binding energies from the interactions at each site, translating into very tight binding.[3b]

Such NRTI/NNRTI double-drugs have used the C-5′, C-5 or N-3 positions of the nucleosides for attachment of the linker. The first examples were reported by the Camarasa[4a] group in 1995 and incorporated a TSAO derivative linked to AZT via a polymethylene spacer between N-3 on each drug. Subsequent work by these authors extended to incorporating d4T as a nucleoside as well as derivatising to form phosphoramidate[4b] double-drugs. Foscarnet was also evaluated as an NNRTI option.[4c] Subsequently, the Ladurée group reported the synthesis and activity of both non-cleavable[5a,b] and cleavable systems[5c] in which the former type as a [d4T]-spacer-[imidazo{1,5-*b*}pyridazine] system was found to be the most active. Similarly, Pedersen[6] recently reported the synthesis and antiviral activities of double-prodrugs incorporating d4T as an *S*-acyl-2-thioethyl (SATE) 5′-phosphate ester linked to N-3 of MKC-442 (a HEPT derivative) via a cleavable *p*-hydroxybenzoyl linker. These double-drugs displayed good anti-HIV activity which was attributed to the d4T part of the molecule, Figure 1.

The objective of the present study was to synthesize and evaluate the anti-HIV activity of bifunctional compounds of the general formula [d4U]-spacer-[HI-236] in the search for evidence of synergism between the

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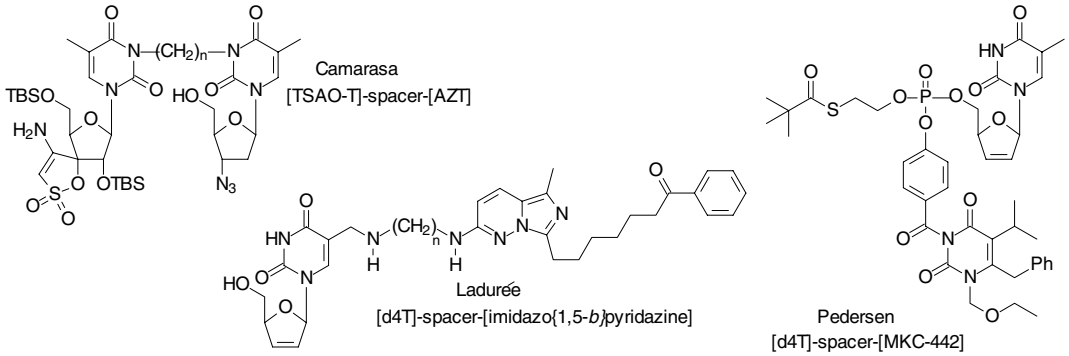
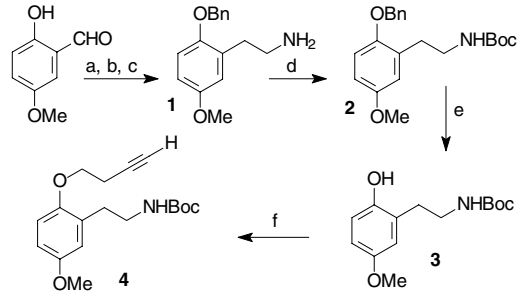


Figure 1. Structures of known NRTI/NNRTI double-drugs.

two drug sites. A biologically non-cleavable butynyl spacer was chosen for the prototype, Figure 2. HI-236 was selected due to its potency against wild-type and NNRTI-resistant HIV-1 strains,[7] while d4T is currently used to treat HIV. Regarding the attachment points for the linker, on the NRTI side, the pyrimidine C-5 position was selected in view of its anticipated low interference with base pairing.[8] On the NNRTI side, attachment to the C-2 phenolic oxygen (see Fig. 2 for numbering) was chosen in view of structural studies reported by Uckun and co-workers,[7] which revealed that the C-2 phenolic oxygen substituent of HI-236 is located beneath the ethyl linker and points into a sizeable hydrophobic space. Attachment of the linker to the C-5 position of the nucleoside was thought to be accessible via a Sonogashira[9] palladium cross-coupling reaction of a terminal alkyne with the 5-iodo nucleoside. During the course of this study, C-5 tethered heterodimers were reported[5c] by the Ladurée group in which a Sonogashira coupling strategy was demonstrated.

Various synthetic disconnections were investigated, and eventually it became clear that the thiourea moiety needed to be introduced last. Thus, the key alkyne **4** was synthesized in six steps starting from commercially available 2-hydroxy-5-methoxybenzaldehyde (Scheme 1), beginning with chemistry described by Glennon[10] and coworkers to afford amine **1**. To assist with isolation and subsequent elaboration, **1** was protected as its Boc-carbamate with *di-tert*-butyldicarbonate and triethylamine in acetonitrile at room temperature to give **2** in 76% yield (over 2 steps) after purification by column chromatography (mp: 103–104 °C). Hydrogenolytic debenzylation of **2** in the presence of 10% palladium-on-carbon in ethanol at room temperature led to phenol



Scheme 1. Reagents and conditions: (a) BnBr, K_2CO_3, EtOH, reflux, 16 h, (95%); (b) CH_3NO_2, NH_4OAc, 70 °C, 14 h, (75%); (c) $LiAlH_4$, THF, reflux, 4 h; (d) $(Boc)_2O$, Et_3N, CH_3CN, rt, overnight, (76%, 2 steps); (e) H_2, Pd/C, EtOH, rt, 5 h, (66%); (f) 3-butynyl-1-tosylate (6 equiv), K_2CO_3 (1.5 equiv), CH_3CN, reflux, 5 d, (70%).

3 in good yield (mp: 116–117 °C; *m/z* HRMS (EI) 267.1433, $C_{14}H_{21}NO_4$ requires *m/z* 267.1471), which was alkylated with 3-butynyl-1-tosylate (6 equiv)[11] using K_2CO_3 (1.5 equiv) in acetonitrile at reflux for 5 days to produce **4** in 70% yield after chromatography (mp: 76–77 °C; *m/z* HRMS(EI) 319.1776, $C_{18}H_{25}NO_4$ requires *m/z* 319.1784).

The Sonogashira coupling partner **6** was synthesized by iodinating 5′-*O*-benzoyl d4U **5**, which was prepared in four steps from uridine according to the Bristol-Myers Squibb procedure for producing d4T from 5-methyluridine.[12] Iodination of **5** was accomplished using elemental iodine and ceric ammonium nitrate (CAN)[13] in acetonitrile at 60 °C in 80% yield (Scheme 2) to afford **6**, which underwent Sonogashira coupling with alkyne **4** to yield the desired coupled product **7** in 69% yield. With **7** in hand, benzoyl deprotection, Boc removal

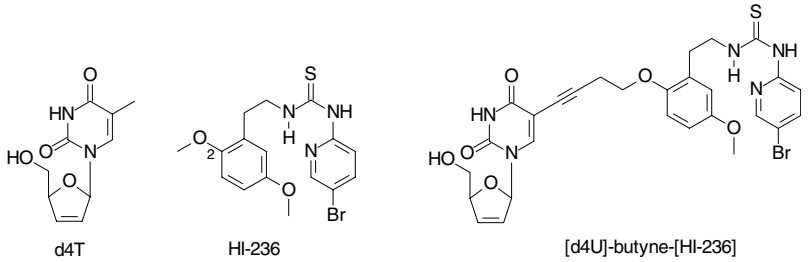
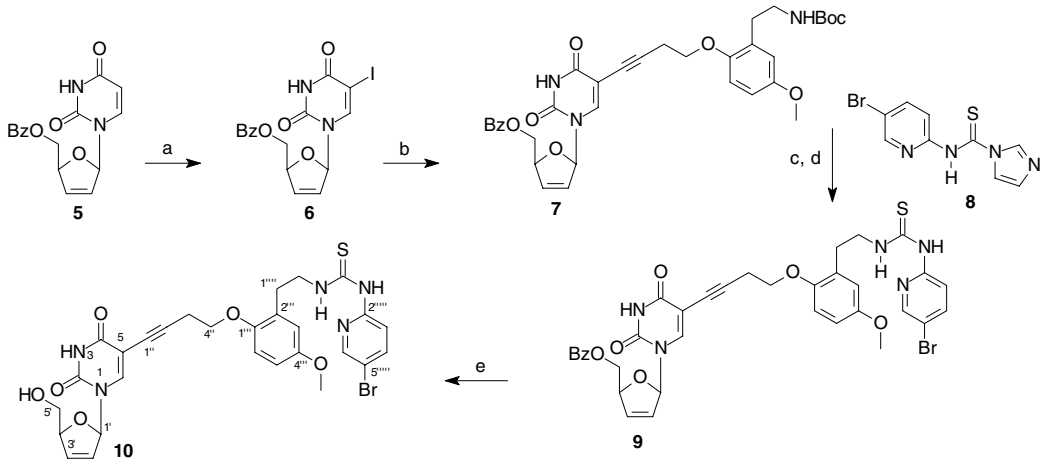


Figure 2. Structures of d4T, HI-236 and [d4U]-butyne-[HI-236].



Scheme 2. Reagents and conditions: (a) I_2, CAN, CH_3CN, 60 °C, 1 h, (80%); (b) alkyne **4**, $(PPh_3)_4Pd$, CuI, Et_3N, DMF, THF, rt, 4 h, (69%); (c) CF_3COOH, CH_2Cl_2, 0 °C; (d) $EtN(i\text{–}Pr)_2$ followed by **8**, THF, rt/overnight, (60% for 2 steps); (e) NaOMe (cat), MeOH, 0 °C, 20 min, (51%).

and thiourea installation remained for completion of the synthesis, and a number of permutations were tried out. Eventually, it was established that debenzoylation as the final step worked best, as the protected alkylated d4U moiety appeared to be less prone to acid-catalysed anomeric cleavage with the trifluoroacetic acid used to deprotect the Boc group. Thus deprotection of the Boc group of **7** using trifluoroacetic acid in methylene chloride at 0 °C furnished the crude amine as a trifluoroacetate salt. Processing involved adding Hünig's base to liberate the amine, and condensing directly with thiocarbonyl derivative **8**[7] at rt overnight in THF. This contrasted with the much harsher conditions used by Sahlberg and co-workers[14] of heating in DMF at 100 °C.

In this fashion, bifunctional benzoate **9** was produced in 60% for the two steps. Final deprotection with catalytic methoxide in methanol furnished the target **10** in 51% yield and in an acceptable level of purity[15] after chromatography.

The inhibition of viral replication in HIV-infected cells of the prototype bifunctional compound **10** together with HI-236, d4T, and the truncated constituent of bifunctional **10**, compound **11**– see Figure 3– were measured against HIV-1 (IIIB) replication in MT-2 cell cultures using an MTT assay.[16] The other truncated component of **10**, compound **12**, has already been evaluated by Ladurée.[17] The results of inhibition are shown in Table 1.

An examination of the inhibitory properties for these compounds offers insight into the design of a prototypical bifunctional compound. Earlier studies from

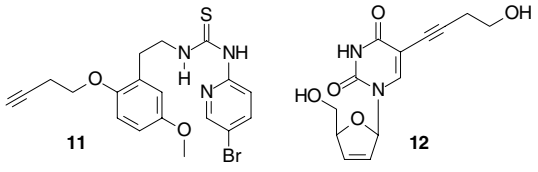


Figure 3. Truncated components of bifunctional **10**.

Table 1. Inhibition and cytotoxicity assay[a] results for **d4T**, **HI-236**, **10**, **11** and **12** against HIV -1 (IIIB) in MT-2 cell culture

Compound	EC_{50}[b] (μM)	Cytotoxicity[c] IC_{50} (μM)	TI[d]
d4T	2	>100	>50
HI-236	0.042	>1	>24
10	0.25	17	68
11	0.023	>1	>44
12[e]	120	360	3

[a] Ref. 16; MOI was 0.1.
[b] Effective concentration that inhibits viral-mediated T-cell death by 50% and as an average of three results.
[c] Concentration that kills 50% of the T-cells and as an average of three results.
[d] In vitro therapeutic index (IC_{50}/EC_{50}).
[e] See Ref. 17.

Laduree's work[17] showed that elongating d4T at C-5 with a hydroxybutynyl spacer (compound **12**) results in a drastic reduction in activity (see Table 1). Interestingly, our current study showed that in elongating the NNRTI, the butynylated HI-236 **11** was found to be twice as active as the parent HI-236. The inhibitory activity of the bifunctional compound **10** as an NRTI-spacer-NNRTI non-cleavable inhibitor is 250 nM. This level of inhibition is eight times greater than that of d4T in this system, and lies between that of HI-236 and d4T, indicating our prototype may be a promising lead for this type of inhibitor. Studies are underway to establish if the NRTI and NNRTI moieties of **10** are indeed simultaneously binding in their respective target sites. The rather potent EC_{50} result for **10** together with the previous results from compound **12**[17] argues that at least a portion of the potential binding interactions for the NRTI sites are being realized. Work is currently in progress to evaluate the influence of changing the spacer as well as derivatising to the C-5′ phosphoramidate.[18]

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.01.107.

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15. Physicochemical characteristics of compounds **10**, [d4U]-butyne-[HI-236], (see Scheme 2 for numbering): mp: 121–122 °C; $[\alpha]_D^{20} + 22.4°$ (*c* 1.10, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3934 (s) (N–H), 3688 (m) (N–H), 2304 (s) (C≡C), 1696 (m) (CO), 1606 (m) (C═C), 1425 (s) (C═S); ^1H NMR (400 MHz, $CDCl_3$) δ: 11.05 (1H, t, *J* = 4.2 Hz, NH), 8.70 (1H, br s, NH), 7.99 (1H, d, *J* = 2.6 Hz, H-6′′′′), 7.97 (1H, s, H-6), 7.56 (1H, dd, *J* = 8.8, 2.6 Hz, H-4′′′′), 6.97 (1H, m, H-1′), 6.80 (1H, m, H-3′′′), 6.77 (2H, m, H-6′′′, H-5′′′), 6.66 (1H, d, *J* = 8.8 Hz, H-3′′′′), 6.37 (1H, dt, *J* = 5.8, 1.5 Hz, H-3′), 5.86 (1H, m, H-2′), 4.93 (1H, m, H-4′), 4.05 (2H, t, *J* = 5.9 Hz, H-4′′), 3.95 (2H, m, H-2′′′′), 3.80 (2H, m, H-5′), 3.78 (3H, s, OMe), 2.98 (2H, m, H-3′′), 2.84 (2H, t, *J* = 5.8 Hz, H-1′′′′); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 178.9 C═S), 162.2 (C-4), 153.4 (C-4′′′), 151.5 (C-2′′′′), 150.9 (C-1′′′), 149.1 (C-2), 146.6 (C-6′′′′), 143.6 (C-6), 140.8 (C-4′′′′), 135.6 (C-3′), 129.3 (C-2′′′), 128.9 (C-2′), 118.0 (C-3′′′), 113.4 (C-3′′′′), 112.8 (C-6′′′), 111.4 (C-5′′′), 110.8(C-5′′′′), 100.3 (C-2′′), 91.3 (C-1′′), 90.4 (C-1′), 87.7 (C-4′), 72.5 (C-5), 66.8 (C-4′′), 62.9 (C-5′), 55.6 (O–Me), 45.7 (C-2′′′′), 30.2 (C-1′′′′), 21.0 (C-3′′); HRMS (ES) *m/z* Found: $[M+H]^+$, 642.1008. Calcd for $C_{28}H_{29}BrN_5O_6S$ (M+H), 642.1022.
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