SYNTHESIS OF 3'-(1,2,3-TRIAZOL-1-YL)-3'-DEOXYTHYMIDINES

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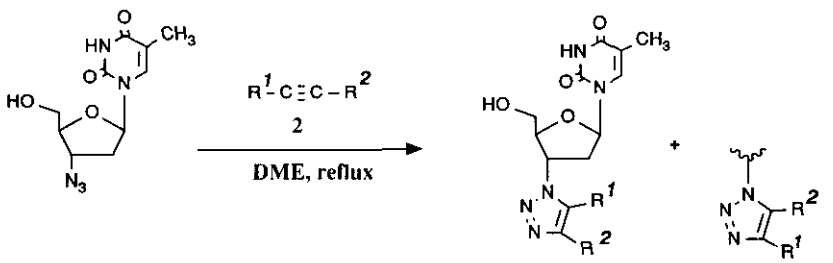
Abstract - The synthesis of various 3'-(1,2,3-triazol-1-yl)-3'-deoxythymidines **3** and their regioisomers **4** by 1,3-dipolar cycloaddition of AZT **1** with alkynes **2** is described.

Unnatural 2',3'-dideoxynucleosides such as 3'-azido-3'-deoxythymidine (AZT, **1**),[1] 3'-deoxy-3'-fluorothymidine,[2] and 2',3'-dideoxycytidine[3] are potent agents against the human immunodeficiency virus (HIV) which gives rise to the acquired immunodeficiency syndrome (AIDS).[4] After conversion to the 5'-triphosphates by cellular kinases, these compounds inhibit the HIV-reverse transcriptase (RT). They act either as competitive inhibitors or as chain terminators of viral DNA-polymerization due to the lack of the 3'-hydroxy group.[5] To date only AZT (**1**) is marketed for AIDS therapy. However, long-term treatment limitations arise from its inherent bone marrow toxicity and the appearance of AZT-resistant mutants,[6] thus making it necessary to search for novel analogs. The azido-function plays an undetermined role in the activity of **1**, which is apparently not limited to its stereoelectronic properties, since several isosters show no significant anti-HIV activity.[2,7]

Our plan was to keep the N_3-unit as part of a triazole ring in order to determine whether its conservation was essential for activity. Tittensor et al. have used cycloadditions of 5'-azido-5'-deoxythymidine with carbonyl activated alkynes to synthesize triazoles as potential thymidylate kinase inhibitors.[8]

Scheme 1:



1 (AZT) **3** **4**

We now describe the synthesis of 3'-(1,2,3-triazol-1-yl)-3'-deoxythymidines **3** and their regioisomers **4** by 1,3-dipolar cycloaddition of AZT (**1**) with alkynes **2** (Scheme 1, Table).[9]

Table:

1,3-Dipolar cycloaddition of AZT (**1**) with alkynes **2** in refluxing DME (Scheme 1)[20]

products no.	R^1	R^2	time (h)	yield[a] (%)	isomers[b] (ratio **3**:**4**)	mp (°C)[c] **3**	**4**
3a	H	$Si(CH_3)_3$	20[d]	92	e	124	
3b	$Si(CH_3)_3$	SO_2Ph	20	63	e	244-246	
3c	$COOCH_3$	$COOCH_3$	1	88	-	foam	
3d	$COOC_2H_5$	$COOC_2H_5$	2	67	-	154	
3e,4e	H	$COOCH_3$	3	91	3:1	f	
3f,4f	H	$COOC_2H_5$	3	89	3:1	125	223
3g,4g	C_2H_5	$COOCH_3$	40	74	1:1	f	
3h,4h	$\underline{n}$-C_3H_7	$COOCH_3$	40	68	1:1	f	
3i,4i	$\underline{n}$-C_5H_{11}	$COOCH_3$	40	70	1:1	f	
3j,4j	$\underline{n}$-C_6H_{13}	$COOCH_3$	40	65	1:1	f	
3k,4k	CH_2OH	$COOCH_3$	20	58	2:1	f	
3l,4l	CH_2OH	COO-PNB[g]	12	90	5:1	f	
3m	CH_2OH	CH_2OH	27	36	-	189	
3n,4n	H	CH_2OH	20	59	3:1	194	186
3o	COOH	COOH	21	83	-	232-234	
3p,4p	H	COOH	20	87	4:1	i	
3q,4q	$\underline{n}$-C_3H_7	COOH	22	68	1:1	f	
3r	COOH	4-NO_2-C_6H_4	15[h]	48	e	>250	
3s	$CONH_2$	$CONH_2$	3[h]	84	-	298	
3t,4t	H		24	82	2:1	212	k
3u,4u	H	4-F-C_6H_4	25[h]	78	2:1	235-236	k
3v,4v	H	2-pyridyl	40	76	2:1	200	234

[a] isolated yield (both isomers)

[b] determined by ^{1}H-nmr

[c] uncorrected

[d] 10 equivalents of TMS-acetylene was used

[e] Only one isomer was isolated after work-up.

[f] Isomers were not separated.

[g] PNB = p-nitrobenzyl

[h] conditions: 110°C in DMF

[i] Separated isomers were isolated as Na salts and lyophilized.

[k] isomer not pure

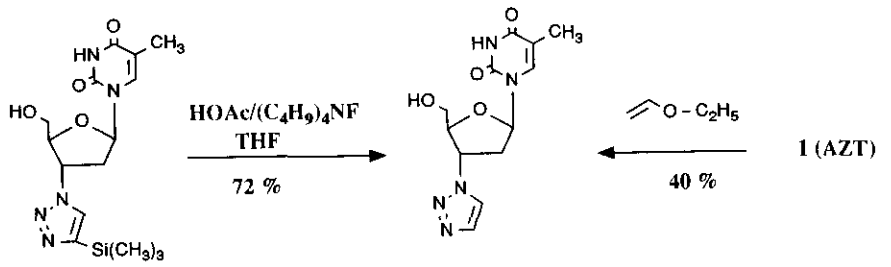
In a **typical procedure** a stirred solution of AZT (**1**) (267 mg, 1.0 mmol) and ethyl propiolate (**2f**) (130 µl, 1.3 mmol) in 2 ml of anhydrous 1,2-dimethoxyethane (DME) was heated to reflux for 3 h. After completion of the reaction (tlc), the solvent

was removed under reduced pressure and the residue was treated with ether to afford 325 mg (89%) of 3:1 mixture of **3f** and **4f**. Chromatography of the mixture on 27 g of silica gel (1:9 toluene/ethyl acetate) gave 51 mg (15%) of the less polar isomer **4f** as crystals, mp 223°C, Rf = 0.19 (1:9 toluene/ethyl acetate) and 231 mg (66%) of the more polar isomer **3f** as crystals, mp 125°C, Rf = 0.09 (same eluent).

The alkynes **2 k**,[10] **2 l**,[11] **2 r**,[12] **2 t**,[13] **2 u**[14] and **2 v**[15] were prepared according to published procedures.

Those unsymmetrical alkynes **2** with substituents R^1 and R^2 of similar steric demand gave intractable 1:1 mixtures of **3** and **4**. Monosubstituted alkynes **2** yielded regioisomers **3** and **4** of markedly different chromatographical and spectroscopical properties, thus permitting easy separation and unambiquous structural assignment. As expected, the sterically less congested isomers **3** were predominant. The more polar, major isomers **3** showed a chracteristic downfield shift of the triazole-H and an upfield shift of the H-3' resonances as compared to the less polar, minor isomers **4**. As a general rule, the chemical shifts of the triazole-H and H-3' differed by more than 3.0 ppm in isomers **3** and by less than 2.5 ppm in isomers **4**. This assignment was confirmed by noe-experiments with both isomers **3n** and **4n**.

Scheme 2:



Protodesilylation[16] of **3a** (5 eq. HOAc/1.5 eq. $(C_4H_9)_4NF$/THF/2.5 h, room temp) afforded triazole **3w** (mp 217°C). This compound could also be synthesized by cyclocondensation[17] of **1** with ethyl vinyl ether (16 h, 100°C) in a pressure vial (Scheme 2). Alkyl substituted triazoles were obtained accordingly by using methyl alkenyl ethers (not shown).

None of the compounds synthesized exhibited appreciable activity in HIV-1 infected CEM-V and MT-2V cells, nor did they inhibit syncytium formation in infected human peripheral blood monocytes.

We have recently compared the X-ray structure of triazole **3w** to other 3'-modified thymidines and have found that the tetrahydrofuran ring of inactive **3w**, contrary to active **1**, adopts a ^{2}E-conformation.[18]

Its inactivity might be due to either lack of phosphorylation by cellular kinases or low affinity of the 5'-triphosphate to the HIV-RT. We have thus synthesized from **3w** the corresponding 5'-triphosphate **5** by modifying a literature procedure[19] [1. $POCl_3/PO(OCH_3)_3$/20 h, 0°C; 2. $[(C_4H_9)_3NH]_2P_2O_7$/DMF/25 min, 0°C; 3. Dowex 50W-X4 (H^+ form); 4. NaOH, pH 7.4] and found that it inhibits HIV-RT in an exogenous assay. It seems as if the reverse transcriptase has less severe steric requirements towards substrates than the thymidine kinases.

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20. All new compounds gave ir, ^{1}H-nmr (300 MHz), mass spectra (ms) and/or combustion analyses consistent with their assigned structures. Selected data: **3a**: Nmr (CD_3OD) δ 0.33 (s, 9H, Si-CH_3); 1.90 (s, 3H, CH_3); 2.75 and 2.90 (m, 2H, H-2'); 3.76 and 3.90 (AB, J=13 Hz, 3.5 Hz, 2H, H-5'); 4.36 (m, 1H, H-4'); 5.46 (m, 1H, H-3'); 6.50 (t, J=7 Hz, 1H, H-1'); 7.92 (s, 1H, H-6); 8.13 (s, 1H, H-5 triazole). Ms (DCI, NH_3) m/z 366 $(M+H)^+$. Uv (MeOH) λ_{max} 264 nm. **3f**: Nmr (DMSO-d_6) δ 1.32 (t, J=7.5 Hz, 3H, $CH_2\underline{CH_3}$); 1.82 (s, 3H, CH_3); 2.6-2.8 (m, 2H, H-2'); 3.78 (m, 2H, H-5'); 4.26 (m, 1H, H-4'); 4.32 (q, J=7.5 Hz, 2H, $\underline{CH_2}CH_3$); 5.78 (t, J=5 Hz, 1H, $CH_2\underline{OH}$); 5.46 (m, 1H, H-3'); 6.43 (t, J=7 Hz, 1H, H-1'); 7.83 (s, 1H, H-6); 8.99 (s, 1H, H-5 triazole); 11.37 (s, 1H, NH). Ms (DCI, NH_3) m/z 366 $(M+H)^+$, 383 $(M+NH_4)^+$.

4f: Nmr (DMSO-d_6) δ 1.33 (t, J=7.5 Hz, 3H, $CH_2\underline{CH_3}$); 1.82 (s, 3H, CH_3); 2.6-2.8 (m, 2H, H-2'); 3.75 (m, 2H, H-5'); 4.3-4.4 (m, 3H, $\underline{CH_2}CH_3$, H-4'); 5.38 (t, J=5 Hz, 1H, $CH_2\underline{OH}$); 5.92 (m, 1H, H-3'); 6.53 (t, J=7 Hz, 1H, H-1'); 7.92 (s, 1H, H-6); 8.35 (s, 1H, H-4 triazole); 11.35 (s, 1H, NH). Ms (DCI, NH_3) m/z 366, $(M+H)^+$.

3s: Nmr (DMSO-$\underline{d}_6$) δ 1.81 (s, 3H, CH_3); 2.6-2.8 (m, 2H, H-2'); 3.75 (m, 2H, H-5'); 4.34 (m, 1H, H-4'); 5.36 (t, J=4.5 Hz, 1H, $CH_2\underline{OH}$); 6.16 (m, 1H, H-3'); 6.55 (t, J=7 Hz, 1H, H-1'); 7.94, 8.16, 8.20, 10.27 (bs, 1H, each, $CONH_2$); 8.50 (s, 1H, H-6); 11.52 (bs, 1H, NH). Ms (FAB) m/z 364 $(M+H)^+$.

3w: Ir (KBr) 3481, 1694, 1471, 1406, 1279, 1138, 1095, 1069 cm^{-1}. Nmr (DMSO-d_6) δ 1.82 (s, 3H, CH_3); 2.6-2.8 (m, 2H, H-2'); 3.62 and 3.72 (AB, J=15 Hz, 4 Hz, 2H, H-5'); 4.21 (m, 1H, H-4'); 5.28 (bs, 1H, OH); 5.41 (m, 1H, H-3'); 6.44 (t, J=7 Hz, 1H, H-1'); 7.82 and 7.84 (s, 2H, H-6, H-triazole); 8.32 (s, 1H, H-triazole); 11.36 (bs, 1H, NH). Ms (EI) m/z 293 $(M)^+$. Uv (MeOH) λ_{max} 206, 264 nm. $[\alpha]_D^{20}$ = -12.6° (c=0.935, MeOH).

5: (Na-salt) Rf=0.05 (4:1 acetonitrile/water): Ir (KBr) 3433, 1701, 1473, 1286, 1158, 1104, 995, 901, 750 cm^{-1}. Nmr (D_2O) δ 2.00 (s, 3H, CH_3); 2.88 (m, 2H, H-2'); 4.32 (m, 2H, H-5'); 4.70 (under solvent H-4'); 5.71 (m, 1H, H-3'); 6.63 (t, J=7 Hz, 1H, H-1'); 7.90 (bs , 2H, H-triazole, H-6); 8.22 (s, 1H, H-triazole). Ms (FAB-) m/z 576 $(M-Na)^-$, 554 $(M-2Na)^-$.

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