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SYNTHESIS OF CHEMICALLY REACTIVE ANALOGUES OF AZT AND THEIR BIOLOGICAL EVALUATION AGAINST HIV

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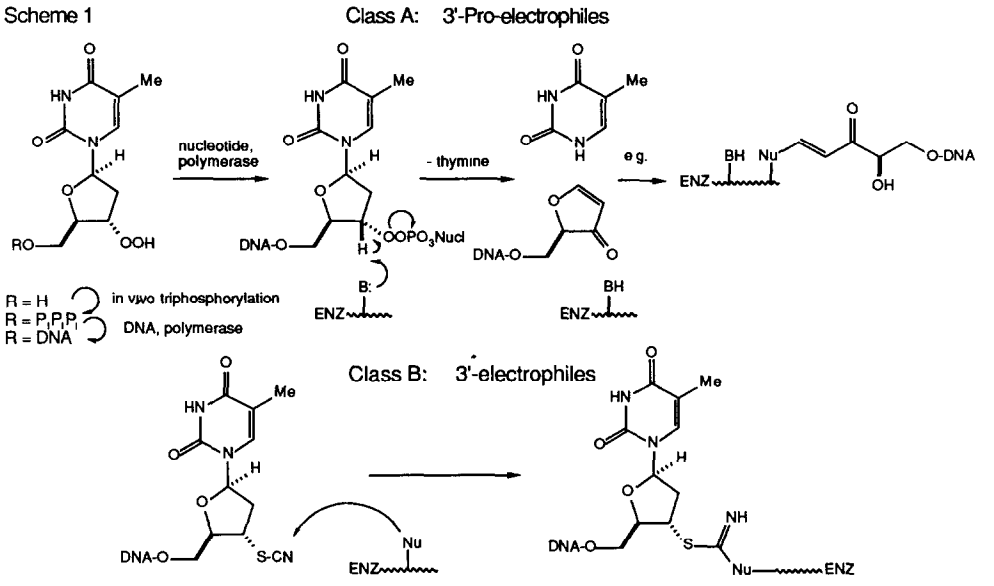
Abstract Several 3'-deoxythymidine analogues were prepared and tested against HIV. The choice of analogues was based on their possible covalent binding to the enzyme reverse transcriptase.

Intensive efforts are underway worldwide to develop chemotherapeutic agents effective against the human immunodeficiency virus (HIV), the etiological agent of acquired immunodeficiency syndrome (AIDS). One common strategy attempts to discover drugs which can interfere with a stage in the viral replicative cycle without damaging the normal processes of the host cell. A prime target is the enzyme reverse transcriptase which catalyzes phosphodiester bond formation on route to the synthesis of a DNA copy of the viral RNA[1]. This enzyme plays a central role in the proliferation of the virus and is not found in the non-invaded host cell. A number of 2',3'-dideoxyribonu-



cleoside analogues that are processed by this enzyme, notably 3'-azido-2',3'-dideoxythymidine (AZT) and 2',3'-dideoxycytidine (DDC),[2] are known to be effective antiretroviral agents. These compounds are incorporated into the nascent DNA chain through the action of reverse transcriptase and act as chain terminators since they lack the 3'-hydroxyl group necessary for continued chain processing.[3]

We have been investigating chemically reactive analogues of AZT that may form a covalent linkage to viral reverse transcriptase. These compounds are equipped with functional groups at the

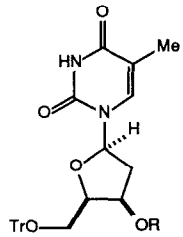
3'-position of the nucleoside that were formulated on consideration of two potential binding modes. The first class of inhibitors (Scheme 1, Class A) is comprised of mechanism-based or "trojan horse" substrates[4] whose reactive functionalities are unmasked as a consequence of their incorporation into the DNA chain. Substrates **4** and **6** (Scheme 2) both possess a 3'-hydroxyl surrogate which could enter into a phosphorylative coupling reaction to further elongate the chain. The phosphorylated product contains a weak bond (RO-OPO_3R', RNH-OPO_3R') whose cleavage would lead to electrophilic intermediates which may react with residues in the active site of the enzyme. Members of the second class of inhibitors (Class B) are equipped with reactive 3'-functional groups that may interfere with the actions of the enzyme by the direct formation of a covalent bond to form a drug-enzyme adduct. Thiocyanate **9** and isothiocyanate **10** were chosen as our preliminary electrophilic targets.[5] Although the nitrile was expected to to serve as a weak electrophile, we included **7** as a candidate structure since a peptidyl nitrile was shown to form a covalent adduct with the cysteine protease papain via a thioimidate ester adduct.[6]

The preparation of the target analogues is illustrated in Scheme 2. The 3'-functional group was introduced by displacement of either the mesylate or the triflate at the 3'-carbon of 5'-O-trityl protected thymidine with the appropriate nucleophile followed by deprotection. Epi-3'-hydroxy-5'-O-tritylthymidine **1** was prepared from thymidine as previously reported[7]. Addition of the mesylate **2**[7] to a mixture of potassium superoxide[8] and 18-crown-6 in DMF at 0°C afforded the hydroperoxide; extended reaction time lead to elimination of the hydroperoxide. Detritylation under acidic conditions afforded 3'-hydroperoxythymidine **4**. The silyl protected peroxide **5** was prepared by protection of the hydroperoxide with *tert*-butyldimethylsilyl triflate. We were concerned that the free hydroperoxide may be susceptible to the *in vivo* action of peroxidophilic agents.[9] Silylation should stabilize the molecule; thus, the ether **5** was hoped to serve as a prodrug (with *in vivo* desilylation). The more reactive triflate **3** was preferred for the preparation of the remaining analogues. Treatment of the alcohol **1** with triflic anhydride followed by direct purification of the reaction mixture by silica gel chromatography afforded the unstable triflate **3** that was used promptly in subsequent transformations. Reaction with O-*tert*-butyldiphenylsilylhydroxylamine,[10] desilylation with tetrabutylammonium fluoride and detritylation with acetic acid provided 3'-hydroxylaminothymidine **6** (HOT). Alternatively, the triflate can be formed and treated *in situ* with the nucleophile. Addition of sodium cyanide to the triflate followed by DMSO (as cosolvent) afforded a 3:2 mixture of nitrile and olefin, which was separated by HPLC (μPorosil, 3:2 hexane/ethyl acetate). Deprotection with pTsOH in methanol afforded 3'-cyano-2',3'-dideoxythymidine **7** and 2',3'-dideoxythymidinene **8**. Similarly, addition of sodium thiocyanate followed by DMF afforded a mixture of thiocyanate and isothiocyanate which were separated by silica gel chromatography and detritylated to afford 3'-thiocyanato-2',3'-dideoxythymidine **9**[2] and 3'-isothiocyanato-2',3'-dideoxythymidine **10**.[11]

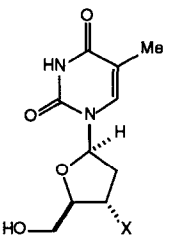
We have modeled the fragmentation of the 3'-peroxyphosphodiester linkage with the peroxyester of **4**. Treatment of the hydroperoxide **11** with acetic anhydride and triethylamine lead to a rapid formation of the furanone **14** with loss of thymine base, presumably *via* the unstable 3'-keto-5'-O-trityl thymidine **13**. Further reaction with excess reagent gave rise to the furan derivative **15**. The furanone could also be prepared conveniently from 5'-O-tritylthymidine **12** by Swern oxidation (oxalyl chloride, Et_3N, DMSO, CH_2Cl_2) through the unstable keto intermediate.[12] The reactivity of the furanone was demonstrated by its facile incorporation of butylamine on route to the vinylogous amide **16**. This reaction is presumed to involve a conjugate addition of the amine to the furanone followed by ring opening and isomerization.

Nucleoside analogues **4-10** were tested for activity against the RF strain of HIV 1 in 8166 cells. Assays were performed in the range of 0.1-100 μg/ml with formation of syncitia at 3-6 days as the endpoint determination. AZT was included as a standard in each test. The hydroperoxide **4**, peroxyether **5** and thiocyanate **9** were weakly active (complete inhibition at ca.100 μg/ml on day 3).

Scheme 2



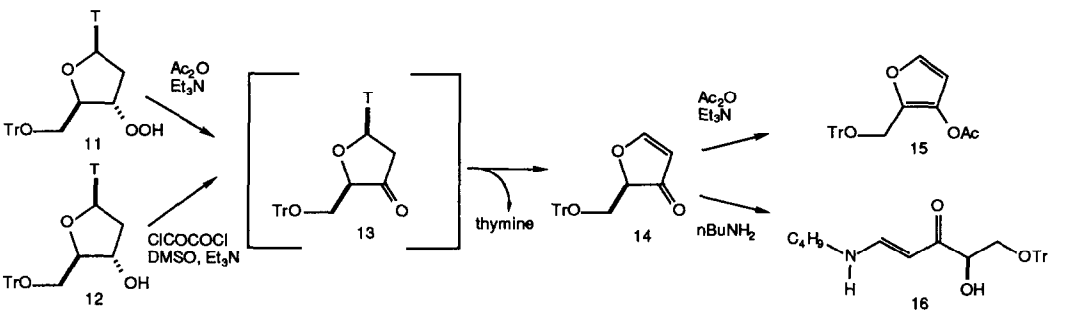
compound	R	precursor	method
1	H	thymidine	a
2	Ms	1	b
3	Tf	1	c



compound	X	precursor	method
4	OOH	2	d
5	OOTBS	2	e
6	NHOH	3	f
7	CN	1	g
8	$\Delta^{2,3}$-ene	1	g
9	SCN	1	h
10	NCS	1	h

Key: [a] 1) 1.1 eq TrCl, py, DMAP, 6h, 80 °C (86%), 2) 1.2 eq MsCl, py, 10h, -4 °C (93%), 3) 1eq NaOH, 2:1 EtOH/H_2O, 10h; 2 eq NaOH, 5h, reflux (86%). [b] 3 eq MsCl, py, 10h, rt (92%). [c] 2 eq Tf_2O, 4 eq py, CH_2Cl_2, 2.5h, 0 °C (76%). [d] 1) 3 eq KO_2, 2 .5 eq 18-C-6, DMF, 30 min, 0 °C (66%), 2) 80% acetic acid, 4 min, 100 °C (76%) [e] 1) d1, 2) 2 eq TBSOTf, 2.2 eq 2,6-lutidine, CH_2Cl_2, 4.5h, rt (80%), 3) 10 eq $ZnBr_2$, CH_2Cl_2, 3h, rt (75%). [f] 1) 2 eq $NH_2OTBDPS$, 1.3 eq $^iPr_2EtN$, CH_2Cl_2, 2 days (52%), 2) 1eq nBu_4NF, THF, 50min, -15 °C (78%), 3) 80% AcOH, 8min, 100 °C (84%). [g] 1) 1.3 eq Tf_2O, 4 eq py, CH_2Cl_2, 2h, 0°C; 10 eq NaCN, DMSO, 30min, 0 °C (71%), 2) 0.5 eq pTsOH, MeOH, 10h, rt (81% (7), 82% (8)). [h] 1) 2 eq Tf_2O, 4 eq py, CH_2Cl_2, 2h, 0 °C; 10 eq NaSCN, DMF, 3h, 0 °C (75% RSCN, 9% RNCS), 2) 0.5 eq pTsOH, MeOH, 3h, rt (76% (9), 60% (10)).

Scheme 3



The isothiocyanate **10** was more active (10 μg/ml on day 3, 50 μg/ml on day 6; compare to AZT: 0.1 μg/ml on day 3, low level syncitia formed at all concentrations on day 6). We note with interest that the nitrile **7** (CN̈T) showed only weak activity (≥ 100 μg/ml), *a finding that is in disagreement with a promising result reported elsewhere.*[13] The olefin **8** had activity comparable to AZT; this result was independently reported during the course of these studies.[14] Preliminary evaluation of the hydroxylamine **6** (HOT) provided encouraging results that warrant further mechanistic and pharmacological investigations. The results of these studies will be reported in due course.

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10. Bottaro, J.C.; Bedford, C.D.; Dodge, A. *Syn. Comm.* **1985**, *15*, 1333. TBDPSCl was substituted for TMSCl; the reported procedure afforded the related reagent in high yield.
11. ^{1}H and ^{13}C NMR spectra were acquired at 250 and 62.8MHz, respectively:
3: ^{1}H NMR($CDCl_3$) 1.82d(1.0,3H), 2.41m(1H), 2.87m(1H), 3.40dd(5.4,10.4,1H), 3.66dd (6.3,10.3,1H), 4.16m(1H), 5.47t(4,1H), 6.26dd(3.1,7.7,1H), 7.18d(1.1,1H), 7.29m(15H), 9.22s(1H). ^{13}C NMR($CDCl_3$) 12.29, 39.77, 61.30, 81.40, 83.71, 85.89, 87.94, 111.60, 127.24, 127.43, 128.01, 128.65, 134.18, 143.23, 150.29, 163.32.
4: $[\alpha]^{24}_D$=+10.5 (c, 2.3 MeOH) ^{1}H NMR(CD_3OD) 1.87d(1.2,3H), 2.2m(1H), 2.5m(1H), 3.73dd(3.4,12.0,1H), 3.82dd(3.0,12.0,1H), 4.18q(1H), 4.64dt(6.6,1.5,1H),6.22dd(5.8,8.8,1H), 7.82d(1.2,1H). ^{13}C NMR(CD_3OD) 12.37, 37.06, 63.64, 84.78, 86.46, 86.51, 111.74, 138.02, 152.36, 166.29.
5: ^{1}H NMR($CDCl_3$) 0.18s(6H), 0.93s(9H), 1.90s(3H), 2.4m(2H), 3.1b(1H), 3.8m(1H), 4.0m(1H), 4.28m(1H), 4.75dm(1H), 6.12dd(6.2,8.5,1H), 7.44s(1H), 9.33s(1H).
6: $[\alpha]^{24}_D$=+13.8 (c,1.56 MeOH). ^{1}H NMR(CD_3OD) 1.88d(1.1,3H), 2.18m(1H), 2.35m(1H), 3.7m(1H), 3.72dd(3.8,1H), 3.84 dd(2.8,12.0,1H), 4.03dd(3.6,6.9,1H), 6.22t(6.9,1H), 7.85d(1.2,1H). ^{13}C NMR(CD_3OD) 12.28, 36.60, 63.37, 63.93, 84.85, 86.79, 111.63, 138.37, 152.53, 166.55.
7: $[\alpha]^{24}_D$= +29.4 (c,1.68 MeOH). IR(KCl plate) 2243cm^{-1}(medium, sharp). ^{1}H NMR (CD_3OD) 1.86d(1.1,3H), 2.56m(1H), 2.73m(1H), 3.51q(9.0,1H), 3.76dd(3.1,12.6,1H), 3.93dd(2.8,12.6,1H), 4.22dt(2.9,8.8,1H), 6.15dd(5.9,7.3,1H), 7.73d(1.1,1H). ^{13}C NMR . (CD_3OD) 12.39, 28.72, 37.15, 61.36, 84.66, 86.78, 111.44, 120.00, 138.21, 152.11, 166.35.
8: $[\alpha]^{24}_D$=+15.5 (c, 2.06 MeOH). ^{1}H NMR(CD_3OD) 1.82d(1.3,3H), 3.75m(2H), 5.90dm(6.1,1H), 6.39dt(1.7,6.1,1H), 6.93m(1H), 7.73d(1.1,1H). ^{13}C NMR(CD_3OD) 12.34, 63.81, 88.93, 91.07, 111.21, 127.27, 35.88, 138.84, 152.84, 166.52.
9: $[\alpha]^{24}_D$= +18.7 (c, 2.11 MeOH). IR (KCl plate) 2155 cm^{-1}(medium, sharp). ^{1}H NMR(CD_3OD) 1.86d(1.1,3H), 2.63m(2H), 3.82dd(2.9,12.6,1H), 3.98m(3H), 6.18dd(4.5,7.0,1H), 7.83d(1.2,1H). ^{13}C NMR(CD_3OD) 12.36, 39.74, 43.65, 61.28, 86.10, 86.87, 111.27, 111.54, 138.17, 152.18, 166.31.
10: IR (KCl plate) 2056 cm^{-1}(strong, broad). ^{1}H NMR(CD_3OD) 1.86d(0.9,3H), 2.53m(2H), 3.75dd(3.2,12.4,1H), 3.85dd(3.0,12.4,1H), 4.08m(1H), 4.55m (1H), 6.22t(6.3,1H), 7.74d(1.0,1H).
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