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Identification of a Novel Family of Nucleosides That Specifically Inhibit HIV-1 Reverse Transcriptase

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Dedicated to Professor Erik De Clercq on his 60th Birthday

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Abstract—*N*-3-Benzyloxycarbonylmethyl- and *N*-3-carboxymethyl-TBDMS-substituted nucleosides were synthesized and evaluated for activity against HIV replication. It was found that the *N*-3-carboxymethyl-TBDMS-substituted nucleosides were specific inhibitors of HIV-1 replication. They should be considered as members of a novel and original class of NNRTIs. © 2001 Elsevier Science Ltd. All rights reserved.

HIV treatment and efficient virus suppression can only be achieved by administration of a concomitant combination of several drugs to HIV-infected individuals. Highly active antiretroviral therapy (HAART)[1] usually consists of at least three, if not more, different anti-HIV drugs. However, HIV chemotherapy has to be improved since residual virus replication is still ongoing under HAART and the compounds can select for drug-resistant virus strains. Thus, novel strategies are required that better suppress or limit the accumulation of resistance mutations in viruses perhaps by, among many other approaches, a faster switching to other drug regimens. Therefore, further development of different types of drugs that may be of use for multiple drug-combination chemotherapy of HIV is needed.

A key target in the search for effective drugs useful for AIDS therapy is reverse transcriptase (RT).[2,3] A number of inhibitors of HIV RT have been developed.[4–6] Among them, the nonnucleoside RT inhibitors (NNRTIs) represent a group of highly potent and specific inhibitors of HIV-1 replication.[7,8] They interact noncompetitively with the enzyme at an allosteric nonsubstrate binding site that is close to but distinct from the polymerase active site.[9–11] Within NNRTIs, TSAO-T[12] is the prototype of a peculiar group of specific HIV-1 RT inhibitors, developed in our laboratories.[12,13] Structurally, TSAO derivatives are highly functionalized nucleosides. These compounds exert their unique selectivity for HIV-1 through a specific interaction with the HIV-1 RT that, unlike the interaction of the nucleoside type of RT inhibitors (i.e., AZT, ddI, d4T, etc.), is noncompetitive with regard to the natural substrates.[14] TSAO derivatives are so far the only family of nucleosides (with an intact sugar moiety) that inhibit HIV-1 replication in a specific manner. TSAO derivatives select for HIV-1 resistance through an unique mutation (E138K) in HIV-1 RT.[15–17]

Following with our efforts to seek novel leads targeted at the HIV-1 RT as specific HIV-1 RT inhibitors, we have identified a novel family of nucleosides that specifically inhibit HIV-1 reverse transcriptase. In this letter, we disclose our synthetic efforts and biological evaluation.

The synthetic routes for the novel specific nucleosides are shown in Schemes 1–3. Reaction of 1-[2′,5′- or 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine[18] (**1** or **2**), 1-[2′,3′,5′-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine[19] (**3**) or 1-[5′-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]thymine[20] (**4**) with benzylbromoacetate, in acetone in the presence of K_2CO_3

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(Scheme 1) gave the corresponding *N*-3-substituted derivatives **5** (90% yield), **6** (73% yield), **7** (71% yield) and **8** (40% yield). Catalytic hydrogenation (H_2, Pd/C) of **5**–**8** gave the corresponding free acid derivatives **9** (82% yield), **10** (85% yield), **11** (76% yield) and **12** (88% yield), respectively.

Treatment of **5** with 80% aqueous acetic acid (Scheme 2) gave the 5′-deprotected analogue **13** (60% yield). Treatment of **5** with tetrabutylammonium fluoride gave the fully deprotected compound **15** (75% yield). Catalytic hydrogenation (H_2, Pd/C) of **13** or **15** gave the carboxymethyl derivatives **14** (74%) and **16** (94%), respectively.

The 3′ or 2′-deoxy- and 2′,3′-dideoxy analogues **18**, **23** and **24** were also prepared. Compound **18** was synthesized by radical deoxygenation from **5**, following the method of Barton and McCombie.[21] Thus, reaction of **5** with *N,N*′-(thiocarbonyl)diimidazole (Scheme 2) in toluene/acetonitrile (1:1), followed by treatment with tributyltin hydride (Bu_3SnH) in the presence of α,α′-azobis(isobutyronitrile), gave the 3′-deoxy derivative **17** in 59% yield. Further treatment of **17** with (H_2, Pd/C) gave the target compound **18** in 85% yield.

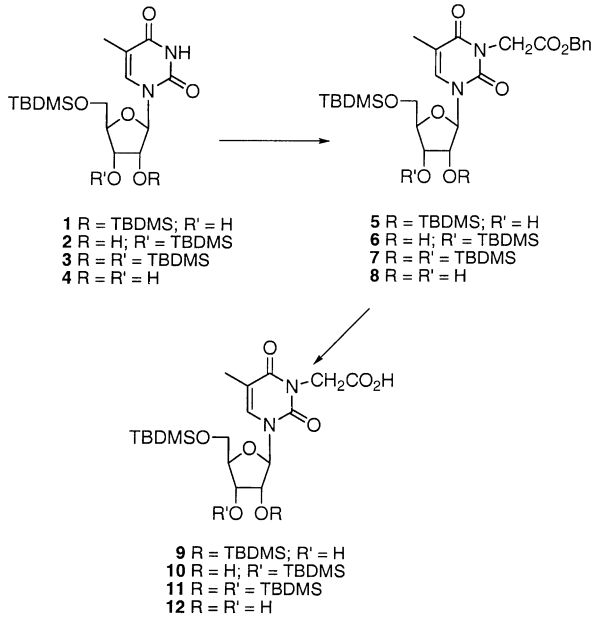
The 2′-deoxy derivative **23** (Scheme 3) was prepared from thymidine as follows: Treatment of 3′,5′-bis-*O*-(*tert*-butyldimethylsilyl)thymidine[22] (**19**) with benzyl bromoacetate in acetone, in the presence of K_2CO_3, gave the *N*-3-substituted derivative **21** (97% yield) which upon catalytic hydrogenation (H_2, Pd/C) gave the 2′-deoxy analogue **23** (81% yield). Finally, the 2′,3′-dideoxy derivative **24** was prepared in 82% yield from 5′-*O*-(*tert*-butyldimethylsilyl)-2′,3′-dideoxythymidine[23] (**20**) following a similar procedure.

Table 1 summarizes the results of the biological evaluation of compounds **5**–**18** and **21**–**24**, expressed as EC_{50} values or compound concentration required to inhibit virus-induced cytopathicity in CEM cell cultures by 50%. The antiviral data on TSAO-T and AZT are also reported as reference compounds.

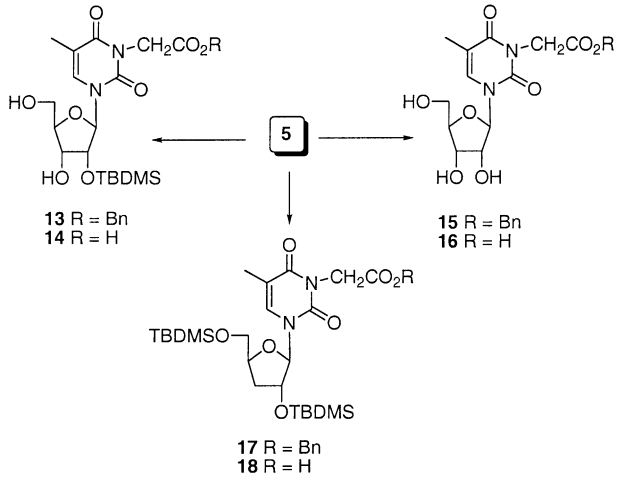
Whereas several compounds inhibited HIV-1 replication in the lower micromolar concentration range, none of the compounds proved active against HIV-2 at subtoxic concentrations (Table 1). Therefore, the active compounds should be considered as specific inhibitors of HIV-1 replication.

Beside of one exception (**13**), none of the *N*-3-benzyl oxycarbonylmethyl derivatives showed antiviral activity whereas the majority of the *N*-3-carboxymethyl derivatives were endowed with anti HIV-1 activity.

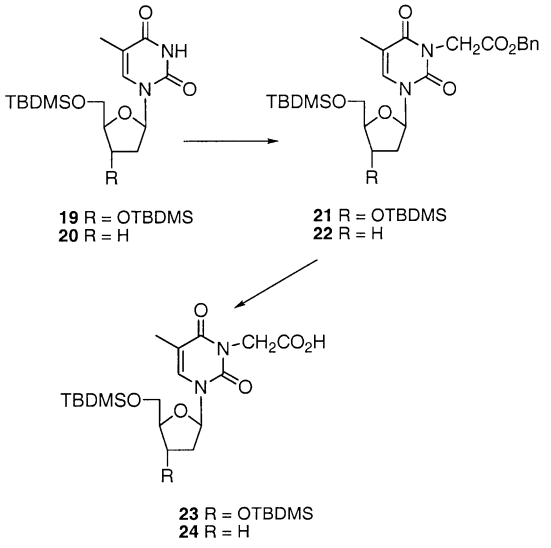
Interestingly, optimal anti HIV-1 activity was achieved with those *N*-3-carboxymethyl thymine nucleosides that contained a TBDMS group at the 5′-position together with one TBDMS group either at 2′ or 3′ of the (deoxy)ribose moiety (**9**, **10**, **18** and **23**).



Scheme 1. Preparation of nucleosides **5**–**12**.



Scheme 2. Preparation of nucleosides **13**–**18**.



Scheme 3. Preparation of nucleosides **21**–**24**.

Table 1. Inhibitory effect of nucleosides **5**–**18**, **21**–**24**, TSAO-T and AZT against HIV-1 and HIV-2 replication in CEM cells[a]

Compds	EC_{50}[b] (μM) HIV-1	EC_{50}[b] (μM) HIV-2	CC_{50}[c] (μM)
5	>10	>10	25.7±2.8
6	>50	>10	57.1±24.3
7	>10	>50	102±9.8
8	>10	>10	30±1.5
9	4.5±2.1	>10	23.0±2.4
10	1.8±2.1	>10	29.3±10.7
11	32±10.6	>125	>125
12	117±41.6	>250	>250
13	4.0±0.0	>10	26±1.4
14	20±7.1	>250	>250
15	>250	>250	>250
16	>250	>250	>250
17	>2	>2	21.6±8.7
18	4.5±0.7	>10	20.7±0.2
21	≥10	>10	56.4±27.5
22	>250	>50	>250
23	5.0±1.4	>10	20.3±0.4
24	>250	>250	>250
TSAO-T	0.06±0.01	>20	14±2
AZT	0.003±0.002	0.004±0.001	6.0±0.1

[a]Data represent the mean values for three independent experiments.
[b]50% Effective concentration, or compound concentration required to inhibit HIV-induced cytopathicity by 50%.
[c]50% Cytotoxic concentration, or compound concentration required to reduce the viability of mock-infected cells by 50%.

Table 2. Sensitivity of HIV-1 wild-type Glu138 and mutant Glu138-Lys recombinant RTs to the inhibitory effect of **9**, **10**, **18** and TSAO-T[a]

Compds	IC_{50}[b] (μM)	
	Glu138 (wild-type HIV-1 RT)	Glu138Lys (mutant HIV-1 RT)
9	138	158
10	207	335
18	33.8	47.4
TSAO-T	3.6	>500

[a]RT reaction was carried out in the presence of poly(C)·oligo(dG) and [^{3}H]dGTP as the template/primer and radiolabeled substrate, respectively.
[b]50% Inhibitory concentration or compound concentration required to inhibit the enzyme activity by 50%.

The presence of only one TBDMS goup at the 5′-position (**12**), or three TBDMS groups at 5′-, 3′- and 2′-position of the ribose (**11**) results in markedly decreased antiviral efficacy. A notable exception was compound **14**, that contained a single TBDMS moiety at the 2′-position of the ribose ring, but still showed moderate anti-HIV-1 activity (Table 1). Although several active compounds showed cytostatic activities between 20 and 100 μM, the viral selectivity amounted up to ≥15 for several compounds, including **10** and **14**.

As demonstrated earlier for the TSAO derivatives, the TBDMS-substituted nucleoside analogues described herein inhibit HIV-1 reverse transcriptase.

However, in contrast with TSAO-T and other TSAO derivatives that contain a 3′-spiro moiety in addition to a TBDMS group at 2′ and 5′ of the ribose ring, the active *N*-3-carboxymethyl nucleoside analogues here described (i.e., **9**, **10**, **18**) keep full inhibitory activity against mutant HIV-1 reverse transcriptase that contain the Glu138Lys mutation (Table 2). These findings are in agreement with our observation that the novel nucleoside analogues keep inhibitory potential against a mutant HIV-1/138Lys strain in cell culture. Indeed, compounds **9**, **10** and **18** showed an EC_{50} against HIV-1/138Lys of 2.0, 8.2 and 4.0 μM, respectively. Our results indicate that the novel nucleoside analogues interact differently with HIV-1 RT than the TSAO derivatives, and thus, *N*-3-carboxymethyl-TBDMS-substituted nucleoside analogues should be considered as members of a novel and original class of NNRTIs.

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